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Antioxidant supplementation for lung disease in cystic fibrosis (Review)

Ciofu O, Smith S, Lykkesfeldt J

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TABLE OF CONTENTS

[Intervention Review]

Antioxidant supplementation for lung disease in cystic fibrosis

Oana Ciofu[1], Sherie Smith[2], Jens Lykkesfeldt[3]

[1]Department of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark. [2]Division of Child Health, Obstetrics & Gynaecology (COG), School of Medicine, University of Nottingham, Nottingham, UK. [3]Department of Veterinary Disease Biology, Experimental Animal Models, University of Copenhagen, Copenhagen, Denmark

Contact address: Oana Ciofu, Department of International Health, Immunology and Microbiology, University of Copenhagen, Blegdamsvej 3, Copenhagen, 2200, Denmark. ociofu@sund.ku.dk.

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ABSTRACT

Background

Airway infection leads to progressive damage of the lungs in cystic fibrosis (CF) and oxidative stress has been implicated in the etiology. Supplementation of antioxidant micronutrients (vitamin E, vitamin C, beta-carotene and selenium) or N-acetylcysteine (NAC) as a source of glutathione, may therefore potentially help maintain an oxidant-antioxidant balance. Glutathione or NAC can also be inhaled and if administered in this way can also have a mucolytic effect besides the antioxidant effect. Current literature suggests a relationship between oxidative status and lung function. This is an update of a previously published review.

Objectives

To synthesise existing knowledge on the effect of antioxidants such as vitamin C, vitamin E, beta-carotene, selenium and glutathione (or NAC as precursor of glutathione) on lung function through inflammatory and oxidative stress markers in people with CF.

Search methods

The Cochrane Cystic Fibrosis and Genetic Disorders Group's Cystic Fibrosis Trials Register and PubMed were searched using detailed search strategies. We contacted authors of included studies and checked reference lists of these studies for additional, potentially relevant studies. We also searched online trials registries.

Last search of Cystic Fibrosis Trials Register: 08 January 2019.

Selection criteria

Randomised and quasi-randomised controlled studies comparing antioxidants as listed above (individually or in combination) in more than a single administration to placebo or standard care in people with CF.

Data collection and analysis

Two authors independently selected studies, extracted data and assessed the risk of bias in the included studies. We contacted study investigators to obtain missing information. If meta-analysed, studies were subgrouped according to supplement, method of administration and the duration of supplementation. We assessed the quality of the evidence using GRADE.

Main results

One quasi-randomised and 19 randomised controlled studies (924 children and adults) were included; 16 studies (n = 639) analysed oral antioxidant supplementation and four analysed inhaled supplements (n = 285). Only one of the 20 included studies was judged to be free of bias.

Oral supplements versus control

The change from baseline in forced expiratory volume in one second (FEV_1) % predicted at three months and six months was only reported for the comparison of NAC to control. Four studies (125 participants) reported at three months; we are uncertain whether NAC improved FEV_1 % predicted as the quality of the evidence was very low, mean difference (MD) 2.83% (95% confidence interval (CI) -2.16 to 7.83). However, at six months two studies (109 participants) showed that NAC probably increased FEV_1 % predicted from baseline (moderate-quality evidence), MD 4.38% (95% CI 0.89 to 7.87). A study of a combined vitamin and selenium supplement (46 participants) reported a greater change from baseline in FEV_1 % predicted in the control group at two months, MD -4.30% (95% CI -5.64 to -2.96). One study (61 participants) found that NAC probably makes little or no difference in the change from baseline in quality of life (QoL) at six months (moderate-quality evidence), standardised mean difference (SMD) -0.03 (95% CI -0.53 to 0.47), but the two-month combined vitamin and selenium study reported a small difference in QoL in favour of the control group, SMD -0.66 (95% CI -1.26 to -0.07). The NAC study reported on the change from baseline in body mass index (BMI) (62 participants) and similarly found that NAC probably made no difference between groups (moderate-quality evidence). One study (69 participants) found that a mixed vitamin and mineral supplement may lead to a slightly lower risk of pulmonary exacerbation at six months than a multivitamin supplement (low-quality evidence). Nine studies (366 participants) provided information on adverse events, but did not find any clear and consistent evidence of differences between treatment or control groups with the quality of the evidence ranging from low to moderate. Studies of β-carotene and vitamin E consistently reported greater plasma levels of the respective antioxidants.

Inhaled supplements versus control

Two studies (258 participants) showed inhaled glutathione probably improves FEV_1 % predicted at three months, MD 3.50% (95% CI 1.38 to 5.62), but not at six months compared to placebo, MD 2.30% (95% CI -0.12 to 4.71) (moderate-quality evidence). The same studies additionally reported an improvement in FEV_1 L in the treated group compared to placebo at both three and six months. One study (153 participants) reported inhaled glutathione probably made little or no difference to the change in QoL from baseline, MD 0.80 (95% CI -1.63 to 3.23) (moderate-quality evidence). No study reported on the change from baseline in BMI at six months, but one study (16 participants) reported at two months and a further study (105 participants) at 12 months; neither study found any difference at either time point. One study (153 participants) reported no difference in the time to the first pulmonary exacerbation at six months. Two studies (223 participants) reported treatment may make little or no difference in adverse events (low-quality evidence), a further study (153 participants) reported that the number of serious adverse events were similar across groups.

Authors' conclusions

With regards to micronutrients, there does not appear to be a positive treatment effect of antioxidant micronutrients on clinical end-points; however, oral supplementation with glutathione showed some benefit to lung function and nutritional status. Based on the available evidence, inhaled and oral glutathione appear to improve lung function, while oral administration decreases oxidative stress; however, due to the very intensive antibiotic treatment and other concurrent treatments that people with CF take, the beneficial effect of antioxidants remains difficult to assess in those with chronic infection without a very large population sample and a long-term study period. Further studies, especially in very young children, using outcome measures such as lung clearance index and the bronchiectasis scores derived from chest scans, with improved focus on study design variables (such as dose levels and timing), and elucidating clear biological pathways by which oxidative stress is involved in CF, are necessary before a firm conclusion regarding effects of antioxidants supplementation can be drawn. The benefit of antioxidants in people with CF who receive CFTR modulators therapies should also be assessed in the future.

PLAIN LANGUAGE SUMMARY

How do vitamins E and C, beta-carotene, selenium and glutathione affect lung disease in people with cystic fibrosis?

Background

Frequent chest infections cause long-term lung inflammation; inflammation-causing cells produce an oxygen molecule (reactive oxygen species (ROS)), which may harm body tissue (oxidative damage); the body uses antioxidants to protect itself. People with cystic fibrosis (CF) have high levels of ROS compared to low levels of antioxidants. Antioxidant supplements might reduce oxidative damage and build up levels of antioxidants.

Given difficulties in absorbing fat, people with CF have low levels of fat-soluble antioxidants (vitamin E and beta-carotene). Water-soluble vitamin C decreases with age in people with CF. Glutathione, one of the most abundant antioxidants in cells, is not released properly into the lungs of people with CF. Some enzymes that help antioxidants work depend on the mineral selenium, so selenium supplements aim to stimulate antioxidant action.

Most supplements are swallowed, but glutathione and N-acetylcysteine (NAC) (which the body uses to make glutathione) can also be inhaled; these may affect lung function as antioxidants, but also due to thinning mucus when inhaled (allowing easier mucus clearance).

Search date

Last search for this updated review: 08 January 2019.

Study characteristics

We included 20 studies (924 people with CF, almost equal gender split, aged six months to 59 years); 16 studies compared oral supplements to placebo ('dummy' treatment) and four compared inhaled supplements to placebo.

Key results

Oral supplements

We are uncertain whether NAC changes lung function (forced expiratory volume in one second (FEV_1) % predicted) at three months (four studies, 125 participants, very low-quality evidence), but at six months two studies (109 participants) reported NAC probably improved FEV_1 % predicted (moderate-quality evidence). One study (46 participants) reported a greater change in FEV_1 % predicted with placebo than with a combined vitamin and selenium supplement after two months. One study (61 participants) reported little or no difference in quality of life (QoL) scores between NAC and control after six months (moderate-quality evidence), but the two-month combined vitamin and selenium study reported slightly better QoL scores in the control group. NAC probably made no difference to body mass index (BMI) (one study, 62 participants, moderate-quality evidence). One study (69 participants) reported that a mixed vitamin and mineral supplement may lead to a lower risk of pulmonary exacerbation at six months than a multivitamin supplement (low-quality evidence). Nine studies (366 participants) did not find any clear and consistent differences in side effects between groups (evidence ranged from low to moderate quality). Vitamin E and β-carotene studies consistently reported greater levels of these antioxidants in blood samples.

Inhaled supplements

In two studies (258 participants), inhaled glutathione probably improved FEV_1 % predicted compared to placebo at three months but not at six months (moderate-quality evidence); these studies also reported a greater improvement in FEV_1 litres with glutathione compared to placebo at both time points. Two studies (258 participants) found little or no difference in the change in QoL scores (moderate-quality evidence). One two-month study (16 participants) and a 12-month study (105 participants) reported no difference between groups in the change in BMI. There was no difference in the time to the first pulmonary exacerbation in one six-month study. Two studies (223 participants) reported no difference between groups in side effects (low-quality evidence) and another study (153 participants) reported that the number of serious side effects were similar across groups.

Conclusions

Vitamin and mineral supplements do not seem to improve clinical outcomes. Inhaled glutathione appears to improve lung function, while oral administration lowers oxidative stress, with benefits to lung function and nutritional measures. Intensive antibiotic and other concurrent treatments for people with CF and chronic infection mean it is difficult to assess the effect of antioxidants without a very large and long study. Future research should look at how antioxidants affect people with CF taking CFTR modulator therapies.

Quality of the evidence

Evidence ranged from very low to moderate quality. All but one study had some bias; mostly because data were not fully reported (likely to affect our results). We were also largely unsure if participants knew which treatment they received, both in advance and once the studies started (unsure how this might affect our results).

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Summary of findings: oral antioxidants (NAC/GSH) compared to placebo

Oral antioxidants (NAC/GSH) compared to placebo for cystic fibrosis

Patient or population: adults and children with cystic fibrosis

Settings: outpatients

Intervention: oral antioxidants NAC/GSH

Comparison: placebo

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	NAC/GSH				
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 3 months	The mean change in FEV_1 % predicted ranged across control groups from -8.6% to -1.64%.	The mean change in FEV_1 % predicted in the intervention groups was 2.83% higher (2.16% lower to 7.83% higher).	NA	125 (4)	⊕⊝⊝⊝ **very low**[1,2,3]	The studies included in this analysis looked at different dosages of NAC. In 2 studies (n = 67), 600 mg/daily was divided in 3 doses (Ratjen 1985; Stafanger 1989), while in the remaining studies the doses were 2700 mg daily (n = 70) (Conrad 2015) and 2800 mg daily (n = 21) (Dauletbaev 2009) divided in 3 and 4 doses, respectively.
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 6 months	Change in FEV_1 was significantly higher in the intervention group compared to the control group, MD 4.38 (95% CI 0.89 to 7.87).		NA	62 (1)	⊕⊕⊕⊝ **moderate**[3]	Results from the only included study favour NAC (2700 mg daily). A further study (n = 47) reported FEV_1 % predicted at 6 months of GSH (administered as L-glutathione), MD 17.40% (95% CI 13.97 to 20.83) (Visca 2015), but the studies were not combined due to the different action of the intervention. Both studies reported in favour of the antioxidant treatment.
Quality of life:	There was no significant difference in the change from from baseline		NA	61 (1)	⊕⊕⊕⊝ **moderate**[3]	Both groups showed a non-significant decrease in CFQ-R score (quality of life worsened) (P = 0.91).

change in mean CFQ-R score from baseline Follow-up: 6 months	in either group for CFQ-R score at 6 months, MD -0.03 (95% CI -0.53 to 0.47).				
Nutritional status: change from baseline in BMI Follow-up: 6 months	There was no significant difference from baseline in either group for BMI score at 6 months, MD 0.20 (95% CI -0.23 to 0.63).	NA	62 (1)	⊕⊕⊕⊖ **moderate**[3]	There was no difference in the change in BMI between the intervention and control group. A further study (n = 47) reported on the change in BMI percentile after 6 months of oral supplementation with GSH (Visca 2015); BMI percentile increased significantly more with GSH supplementation than control, MD 17.20% (95% CI 14.35 to 20.05).
Pulmonary exacerbations: mean time to next exacerbation Follow-up: 6 months	Outcome not reported.				Although time to exacerbation was not reported, the Conrad study (n = 70) reported no difference in the number of participants with pulmonary exacerbations requiring antibiotics between NAC and control at 6 months, RR 0.83 (95% CI 0.50 to 1.390) and also reported no difference in the number of participants hospitalised, RR 0.94 (95% CI 0.49 to 1.81) (Conrad 2015).
Adverse events Follow-up: 6 months	Reported adverse effects were more common in the intervention group for: sinusitis, OR 2.92 (95% CI 0.11 to 74.05); diarrhoea, OR 1.29 (95% CI 0.27 to 6.25); and elevated liver enzymes, OR 2.92 (95% CI 0.11 to 74.05); but were more common in the control group for distal intestinal obstruction syndrome, OR 0.31 (95% CI 0.01 to 7.77).		70 (1)	⊕⊕⊕⊖ **moderate**[3]	Two further studies (n = 41) reported no adverse events in either control or intervention group (Götz 1980; Mitchell 1982). One study (n = 21) reported an adverse effect (gastrointestinal bleeding) which was considered to have a "possible" relationship to the medication (Dauletbaev 2009).

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
BMI: body mass index; **CI**: confidence interval; **FEV_1**: forced expiratory volume in 1 second; **GSH**: glutathione; **MD**: mean difference; **NAC**: N-acetylcysteine; **OR**: odds ratio; **RR**: risk ratio.

GRADE Working Group grades of evidence
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: we are very uncertain about the estimate.

1. Downgraded once due to risk of bias across the included studies for this outcome; 1 study had a low risk of bias overall, but the remaining 3 had an unclear or high risk of bias across some of the domains particularly around allocation concealment and blinding.
2. Downgraded once due to inconsistency of results. There was heterogeneity with an I^2 value of 49% across the studies; this may be due to the different doses of NAC given and the quality of the underlying studies.
3. Downgraded once due to imprecision from small participant numbers.

Summary of findings 2. Summary of findings: oral vitamin E supplementation versus placebo or no treatment

Oral antioxidant vitamin E supplement compared with placebo or no treatment for cystic fibrosis

Patient or population: children and adults with cystic fibrosis

Settings: outpatients

Intervention: oral antioxidant vitamin E supplement

Comparison: placebo or no treatment

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo/no treatment	Vitamin E supplement				
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 3 months	Outcome not reported.					
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 6 months	Outcome not reported.					
Quality of life Follow-up: 6 months	Outcome not reported.					
Nutritional status: change from baseline in BMI Follow-up: 6 months	Outcome not reported.					

Pulmonary exacerbations: mean time to next exacerbation Follow-up: 6 months	Outcome not reported.			
Adverse events Follow-up: 3 months	No difference was found in the number of adverse events due to: sinusitis, OR 1.00 (95% CI 0.13 to 7.94); or exacerbations OR 1.00 (95% CI 0.06 to 17.25). Reported adverse effects were more common in the intervention group for: distal intestinal obstruction syndrome, OR 3.16 (95% CI 0.12 to 82.64); diarrhoea, OR 3.16 (95% CI 0.12 to 82.64); and elevated liver enzymes, OR 3.16 (95% CI 0.12 to 82.64).	38 (1)	⊕⊕⊖⊖ **low**[1,2]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
BMI: body mass index; **CI**: confidence interval; **FEV_1**: forced expiratory volume in 1 second; **OR**: odds ratio.

GRADE Working Group grades of evidence
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: we are very uncertain about the estimate.

1. Downgraded once due to risk of bias within the included study across the domains of randomisation, allocation concealment, incomplete outcome reporting and selective reporting.
2. Downgraded due to low numbers of participants and small number of participants.

Summary of findings 3. Summary of findings: oral β-carotene compared to placebo

Oral antioxidant β-carotene compared to placebo for cystic fibrosis

Patient or population: children and adults with cystic fibrosis

Settings: outpatients

Intervention: oral antioxidant β-carotene

Comparison: placebo

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				

	Placebo	β-carotene				
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 3 months	Outcome not reported.					
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 6 months	There was no significant difference from baseline in either group for FEV_1 % predicted at 6 months, MD 0.90 (-20.09 to 21.89).		NA	24 (1)	⊕⊖⊖⊖ **very low[1,2,3]**	P = 0.93
Quality of life Follow-up: 6 months	Outcome not reported.					
Nutritional status: change from baseline in BMI Follow-up: 6 months	Outcome not reported.					
Pulmonary exacerbations: mean time to next exacerbation Follow-up: 6 months	Outcome not reported.					
Adverse events Follow-up: 6 months	The study reported no adverse events in either group.		NA	24 (1)	⊕⊖⊖⊖ **very low[1,2,3]**	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
BMI: body mass index; **CI**: confidence interval; **FEV_1**: forced expiratory volume in 1 second; **MD**: mean difference.

GRADE Working Group grades of evidence
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: we are very uncertain about the estimate.

1. Downgraded once due to unclear or high risk of bias across most domains, particularly randomisation, allocation concealment and publication bias.
2. Downgraded once due to imprecision from a small sample size.
3. Downgraded once because of a high risk of publication bias with the study being published multiple times without reference to other publications.

Summary of findings 4. Summary of findings: oral antioxidant combination compared to control

Oral antioxidant combination compared with control for cystic fibrosis

Patient or population: children with cystic fibrosis

Settings: outpatients

Intervention: oral antioxidant combination (vitamins E, C, A, β-carotene and selenium)

Comparison: control (continuation of a low-dose supplement)

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control (continuation of low dose supplement)	Combination of oral antioxidants				
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 3 months	Outcome not reported at this time point.					After 2 months of a combined supplement, a single study reported a significant difference in favour of control, MD -4.30% (95% CI -5.64 to -2.96).
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 6 months	Outcome not reported.					
Quality of life:	Outcome not reported at this time point.					Results significantly favoured control over antioxidant supplementation at 2 months, SMD

Quality of Well Being score change from baseline Follow-up: six months		-0.66 points (95% CI -1.26 to -0.07). A higher score indicates better quality of life.
Nutritional status: change from baseline in BMI Follow-up: 6 months	Outcome not reported.	
Pulmonary exacerbations: mean time to next exacerbation Follow-up: 6 months	Outcome not reported.	
Adverse events Follow-up: 6 months	Outcome not reported.	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
BMI: body mass index; **CI**: confidence interval; **FEV_1**: forced expiratory volume in 1 second; **MD**: mean difference; **SMD**: standardised mean difference.

GRADE Working Group grades of evidence
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: we are very uncertain about the estimate.

Summary of findings 5. Summary of findings: oral antioxidant mixed supplement compared with control

Oral antioxidant mixed supplement compared with control for cystic fibrosis

Patient or population: adults and children with cystic fibrosis

Settings: outpatients

Intervention: AquADEKs-2 containing standard amounts of fat-soluble vitamins (A, D, E, K) as in typical CF multivitamin supplements plus several antioxidants including β-carotene, mixed tocopherols (different forms of vitamin E), CoQ10, mixed carotenoids (lutein, lycopene and zeaxanthin), and the minerals zinc and selenium

Comparison: control multivitamin softgel capsules

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control capsules	Mixed supplement				
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 3 months	Outcome not reported at this time point.					At 4 months the MD between the groups was 1.44 higher (95% CI -2.23 to 5.11) favouring the mixed supplement. The effect was not significant P = 0.44.
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 6 months	Outcome not reported.					
Quality of life: Quality of Well Being score change from baseline Follow-up: 6 months	Outcome not reported.					
Nutritional status: change from baseline in BMI Follow up: 6 months	Outcome not reported at this time point.					The study did however report that at 4 months there was no difference in weight z scores between the intervention and control group (Sagel 2018).
Pulmonary exacerbations: mean time to next exacerbation Follow-up: 6 months	There was a significantly lower risk of first pulmonary exacerbation in the antioxidant group than the control group at 4 months, HR 0.5 (95% CI 0.25 to 0.98) (P = 0.04).		NA	69 (1)	⊕⊕⊖⊖ **low[1,2]**	This result was reported directly from the paper.
Adverse events Follow-up: 6 months	No significant differences were found in the number of adverse events due to: sinusitis, OR 2.12 (95% CI 0.18 to 24.44); DIOS, OR 0.24 (95% CI 0.03 to 2.22); diarrhoea, OR		NA	69 (1)	⊕⊕⊖⊖ **low[1,2]**	Although not statistically significant, adverse events were more common in the intervention group for sinusitis and diarrhoea whilst they were

	2.19 (95% CI 0.37 to 12.76); or pulmonary exacerbations, OR 0.54 (95% CI 0.21 to 1.39).	more common in the control group for DIOS and pulmonary exacerbations.

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
BMI: body mass index; **CI**: confidence interval; **DIOS**: distal intestinal obstruction syndrome; **FEV_1**: forced expiratory volume in 1 second; **HR**: hazard ratio; **MD**: mean difference; **OR** odds ratio.

GRADE Working Group grades of evidence
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: we are very uncertain about the estimate.

1. Downgraded once due to risk of bias within the single study. There were concerns about allocation concealment and also because the enrolment number was not reached.
2. Downgraded once due to imprecision from low event rates.

Summary of findings 6. Summary of findings: inhaled antioxidants compared with placebo

Inhaled antioxidants compared with placebo for cystic fibrosis

Patient or population: children and adults with cystic fibrosis

Settings: outpatients

Intervention: nebulised GSH

Comparison: placebo

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Nebulised GSH				
Lung function: FEV_1 % predicted mean change from baseline	The mean change in FEV_1 % predicted ranged across control groups from	The mean change in FEV_1 % predicted in the intervention groups was 3.5% higher	NA	258 (3)	⊕⊕⊕⊖ **moderate**[1]	Data significantly favoured the antioxidant group (P = 0.001). This effect remained when the pediatric data were removed from the analysis.

Follow-up: 3 months	-3.54% to 0.32%.	(1.38% higher to 5.62% higher).				
Lung function: FEV_1 % predicted mean change from baseline Follow-up: 6 months	The mean change in FEV_1 % predicted ranged across control groups from -4.18% to 1.53%.	The mean FEV_1 % predicted in the intervention groups was 2.3% higher (0.12% lower to 4.71% higher).	NA	258 (3)	⊕⊕⊕⊖ **moderate**[1]	These results include adult and pediatric data. The adult-only data were also analysed, but there was no significant difference between groups, MD 2.17% (95% CI -1.07 to 5.41).
Quality of life: change in CFQoL score from baseline Follow-up: 6 months	There was no difference between groups MD 0.80 (95% CI -1.63 to 3.23) (P = 0.52).		NA	153 (1)	⊕⊕⊕⊖ **moderate**[2]	2 further studies found no significant difference between groups at 12 months (data taken from the papers) (Calabrese 2015a; Calabrese 2015b).
Nutritional status: change from baseline in BMI Follow-up: 6 months	Outcome not reported at this time point. See comment.					No statistically significant difference was found between groups with regard to BMI either at 2 months, MD 0.10 (95% CI -0.74 to 0.94) or at 12 months, MD 0.04 (95% CI -8.20 to 8.27).
Pulmonary exacerbations: mean time to next exacerbation Follow-up: 6 months	Outcome not reported at this time point. See comment.					The time to first exacerbation was 163 days in the GSH treated group and 141 days in the control group. This difference was reported as not statistically significant by the authors using Wilcoxon rank sum test; not directly analysed in this review due to skewed data.
Adverse events Follow-up: 6 months	No significant differences were seen between groups for any adverse events.		NA	223 (3)	⊕⊕⊖⊖ **low**[1,3]	1 study reported no serious adverse events (Bishop 2005). 2 studies reported that none of the reported adverse events led to discontinuation of the drug and that no death occurred (Calabrese 2015a; Calabrese 2015b). A further study reported that the number of serious adverse events were similar between the group treated with GSH inhalations and the place-

bo group (11% and 10%, respectively) (Griese 2013).

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
BMI: body mass index; **CFQoL**: cystic fibrosis quality of life; **CI:** confidence interval; **FEV_1**: forced expiratory volume in 1 second; **GSH**: glutathione; **MD**: mean difference.

GRADE Working Group grades of evidence
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: we are very uncertain about the estimate.

1. Downgraded once due to risk of bias in the included studies, particularly through lack of blinding caused by the intervention having a distinctive taste and smell.
2. Downgraded once due to risk of bias within the single included study for this outcome. The study was at high risk of bias in the blinding domain as the intervention has a distinctive taste and smell. The participants were also allowed to continue oral N-acetylcysteine (NAC) (a precursor of GSH).
3. Downgraded once due to imprecision caused by low event rates for many of the reported adverse events.

BACKGROUND

Description of the condition

Cystic fibrosis (CF) is the most prevalent inherited, life-limiting disorder in white populations affecting approximately one in 2000 births. It is estimated that the present number of CF cases is 35,000 in Europe, 30,000 in the USA and 3000 in Canada (CCFF 2002; CFF 2005). Approximately 1000 new cases of CF are diagnosed in the USA each year. The median predicted lifespan for people with CF has risen steadily over the last 25 years. Since 2002, the median predicted survival age has increased by almost 10 years, from 31.3 years in 2002 to 41.7 years in 2015 (CFF 2015). Most people with CF are diagnosed before the age of two years. Today, in several countries, CF is typically diagnosed shortly after birth through newborn screening programs, e.g. since 2010, all newborns have been screened for CF in the USA. Early diagnosis may have played an important role in improving survival and research shows that people with CF who are diagnosed through the newborn screening programs have a higher weight and healthier lungs later in life than those diagnosed at a later time point because of CF symptoms (CFF 2015).

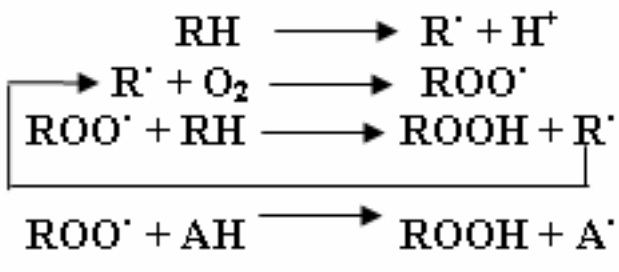
There are more than 1500 mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR) on chromosome 7, which lead to a malfunction of the chloride channel in people with CF. This malfunctioning of the chloride channel in people with CF leads to a decreased volume of the periciliary fluid in the lower respiratory tract, which in turn leads to impaired mucociliary clearance of inhaled microbes. The impaired mucociliary clearance was also proposed to be due to 'sticky', unfolded mucins caused by the lack of bicarbonate ion ($HCO3^{-}$) in the periciliary fluid, as a consequence of the defective CFTR (Quinton 2017). The impairment of the non-inflammatory defence mechanism of the respiratory tract leads to early recruitment of inflammatory defence mechanisms such as polymorphonuclear leukocytes (PMN) and antibodies. However, in spite of an inflammatory response and intensive antibiotic therapy, infections caused by particularly *Pseudomonas aeruginosa* persist and lead to respiratory failure or death. This pathogen is able to survive by switching to the biofilm mode of growth, which provides tolerance to the inflammatory defence mechanisms and antibiotic treatment.

Therefore, from early childhood, people with CF have recurrent and chronic respiratory tract infections characterised by PMN inflammation. Counts of PMNs in CF airway fluid have been found to be thousands of times higher than normal. A consequence of the PMN-dominated inflammation is the release of proteases and reactive oxygen species (ROS), which are believed to be the main modulators of tissue damage in CF. Besides the increased production of ROS, people with CF have an impaired absorption of dietary antioxidants in the gut and the inability of cells bearing mutant CFTR to efflux glutathione (GSH) - the most abundant intracellular antioxidant - into the extracellular milieu of the lung. It has also been shown that concentration of GSH is low in the airways of individuals with CF from an early age (Dickerhof 2017) and that increased oxidation of GSH by neutrophil-derived hypochlorous acid contributes to this deficiency (Kettle 2014). As a primary water-soluble antioxidant, GSH performs several important functions in the epithelial lining fluid by directly scavenging hydrogen peroxide and other free radicals (Kelly 1999). In this process, GSH is oxidized to glutathione disulfide (GSSG).

Furthermore, GSH also plays multiple, pivotal roles in the immune system as normal intracellular levels of GSH are essential for chemotaxis, phagocytosis, oxidative burst etc. In CF, reduced levels of total GSH (GSH and GSSG) and an increased GSSG to GSH redox ratio may partly explain the chronic and excessive inflammation in the respiratory system.

Thus, in CF, the source of oxidative stress is due to the imbalance between increased ROS production and impaired antioxidant systems. It is thought that ROS, which are the key players in oxidative stress, cause tissue damage in the lungs by attacking e.g. polyunsaturated fatty acids (PUFAs) in cell membranes. These PUFAs are one of the main components of dietary fats and are converted to arachidonic acid, a component of phospholipids in cell membranes. It is thought that ROS attack phospholipids (peroxidation) and produce a free radical, which in turn initiates attacks on adjacent arachidonic acid chains, thus compromising cell-membrane structure. Free radical damage is propagated until host defence systems counteracts and terminates these actions. The peroxidation products of arachidonic acid are F_2-isoprostanes and these have become the gold-standard indicator of oxidative stress in vivo (Mayne 2003). The mechanism of peroxide generation, propagation and termination is shown in the figures (Figure 1).

Figure 1. Peroxide chain reaction characterized by initiation, propagation and termination. (RH: PUFA; R·: free radical; ROO·: peroxide; ROOH: hydroxyl peroxide; AH: vitamin E; A·: oxidized Vitamin E. *Adapted from: Tappel AL. Vitamin E and free radical peroxidation of lipids. Annals of the New York Academy of Sciences. 1972; 203(1):12-28.*



Description of the intervention

Unusually high levels of oxidative stress in CF (due to the chronic neutrophilic inflammation in the lungs of people with chronic infection) deplete the host-defence system, which includes exogenous antioxidant micronutrients vitamin E, vitamin C, β-carotene and selenium and the major cellular antioxidant, GSH. Supplementation of these micronutrients or of GSH or N-acetylcysteine (NAC), alternatively referred to as free-radical scavengers, may help in preventing the unfavourable shift towards redox imbalance observed in people with CF.

The ability of a substance to act as an antioxidant in a biologically relevant situation is a highly complex concept. In this respect, the location of ROS generation, the ROS species generated, the relative abundance of endogenous antioxidants in the locality, the rate constants of endogenous antioxidants for the ROS generated, together with their relative concentrations, will all be vital determinants of the success or failure of an administered antioxidant in helping to prevent cellular damage (Rushworth 2013).

Since the CFTR channel is the major mechanism of GSH efflux into the extracellular milieu of the lung from lung epithelial cells, this efflux is severely compromised in CF resulting in GSH system dysfunction. People with CF experience GSH deficiency both locally in the epithelial lining fluid of the lung and also as a systemic GSH deficiency in blood (Roum 1993). Besides this CFTR-related mechanism, the GSH depletion in the CF lung is also caused by oxidation of GSH by myeloperoxidase-derived hypochlorous acid liberated by the neutrophils infiltrating the lungs and it has been shown that this occurs very early in life, already in infancy in people with CF (Kettle 2014).

Pilot studies have shown that it is possible to replete alveolar GSH after GSH inhalation therapy and several clinical studies employing GSH, or a GSH precursor such as NAC, as an intervention have resulted in improved clinically-relevant markers in CF (Tirouvanziam 2006). Therefore, both local treatment (inhalations) and systemic administration (oral) of GSH or the GSH precursor have been proposed to be beneficial for people with CF.

Although many other antioxidants exist, vitamin E, vitamin C, β-carotene, selenium and glutathione or NAC have been chosen in this review due to their well-defined antioxidant properties, mechanisms of action and long history of study in the body (Rock 1996). Other, more recently proposed antioxidants include, for example, other carotenoids (lycopene, zeaxanthin, lutein), melatonin and retinol (Pryor 2000). People with CF are largely affected by pancreatic insufficiency such that, despite replacement pancreatic enzyme therapy and high-fat diets, the absorption of fat and fat-soluble vitamins is usually sub-optimal. Lowered plasma antioxidant status of vitamin C and decreased activity of erythrocyte glutathione peroxidase (GSHPx), an antioxidant enzyme dependent on the mineral selenium, have also been reported in people with CF (Benabdeslam 1999; Wood 2001). The effect of supplementation with vitamin E in CF has already been reviewed in a Cochrane Review (Okebukola 2017). As such, vitamins E and C, β-carotene and selenium as well as glutathione comprise the antioxidant interventions that will be assessed in this review; as their mechanisms of action are sufficiently different, they are subgrouped accordingly.

How the intervention might work

Literature suggests that a relationship exists between oxidative stress status and lung function. Specifically, elevated levels of oxidative stress and inflammatory stress indicators with corresponding reduced lung function have previously been found in individuals with CF (Brown 1994; Brown 1996; Mayer-Hamblett 2007; Wood 2001). Such indicators (oxidative and inflammatory markers) are often used as surrogate outcomes of lung function in respiratory research (Montuschi 1998; Repine 1997; Schunemann 1997). Lung function status or improvements, or both, are also routinely reported in the literature. Due to the chronic and progressive nature of CF, clinical benefits of antioxidant therapy may be difficult to determine.

It is suggested that oral administration of GSH is not optimal due to its poor bioavailability and rapid oxidation (Schmitt 2015). NAC is a thiol and mucolytic agent, a precursor of L-cysteine and reduced GSH. A Cochrane Review looking at nebulised and oral thiol derivatives for pulmonary disease in cystic fibrosis was published in 2013 (Tam 2013). The pharmacokinetics and bioavailability of NAC is highly dependent of the administration route; NAC is rapidly absorbed following oral administration. After absorption, NAC is rapidly metabolised to cysteine, which is a direct precursor in the synthesis of intracellular GSH and in this way, it acts as an antioxidant by restoring the pool of intracellular reduced GSH (Rushworth 2013). This underlines that in order for oral NAC to confer antioxidant activity, the intracellular levels of GSH have to be depleted. There is no detectable NAC in bronchoalveolar lavage after oral administration, therefore it has no mucolytic effect. High-dose oral NAC increases neutrophil GSH levels, decreases airway neutrophil recruitment and most likely act by reducing pulmonary oxidative stress and inflammation (Tam 2013).

Inhaled NAC acts directly on airway secretions and therefore has a mucolytic effect by reducing disulfide bonds (S-S) between glycoproteins in mucus to sulfhydryl bonds (-SH), which no longer participate in cross-linking.

Thus, the potential effect of NAC after oral administration is due to its antioxidant effect, as recently shown in people with CF (Skov 2014), while the effect after inhalation may be multifactorial involving a mucolytic component. Recently, it has been proposed that the effect of GSH inhalations might depend on the sputum levels of gamma-glutamyltransferase, an enzyme that degrades GSH and investigators suggest sputum gamma-glutamyltransferase as a possible biomarker for individualized GSH inhalation treatment in people with CF (Corti 2017).

Why it is important to do this review

A synthesis of all available clinical studies on the effects of antioxidants on lung disease will indicate the relevance of antioxidants to health status in people with CF and will guide future therapeutic decisions. Currently, fat-soluble vitamins (vitamins A, D, E and K) are routinely supplemented in CF to prevent deficiencies associated with fat malabsorption; however, the therapeutic use of antioxidants, such as vitamins C and E, β-carotene, selenium and glutathione is limited. Vitamin A and beta-carotene supplementation is the subject of a Cochrane Review, which aimed to establish whether supplementation reduced the frequency of vitamin A deficiency disorders, improved general and respiratory health or increased the frequency of vitamin A

toxicity; the review identified a single study of beta-carotene supplementation (de Vries 2018). Reviews of vitamin D, vitamin E and vitamin K supplementation have also been published (Ferguson 2014; Jagannath 2015; Okebukola 2017). The present review aims to establish whether antioxidant oral supplementation with micronutrients such as vitamins C and E, β-carotene, selenium or with glutathione (and NAC as precursor to GSH) or inhalation supplementation with GSH are promising adjunct therapies in CF. This is an update of a previously published review (Ciofu 2014; Shamseer 2010).

OBJECTIVES

The objective of the review is to synthesise existing knowledge on the effect of antioxidants such as vitamin C, vitamin E, beta-carotene, selenium and GSH (or N-acetylcysteine as precursor of GSH) on lung function through inflammatory and oxidative stress markers in people with CF.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials (RCTs) and quasi-RCTs.

Types of participants

People of either gender diagnosed with CF and with all degrees of severity (Pellegrino 2005), including those who have undergone lung transplant.

Types of interventions

The interventions considered were antioxidants including vitamin E, vitamin C, β-carotene, selenium and GSH or NAC (as a source of GSH) in more than a single administration, by any route of administration and solubility taken individually or in combination compared to placebo or standard medication or care.

Types of outcome measures

Primary outcomes

1. Lung function tests
   a. forced expiratory volume in one second (FEV_1) (% predicted or L)
   b. forced vital capacity (FVC) (% predicted or L)
2. Quality of life (QoL) (using validated measurement tools only)

Secondary outcomes

1. Oxidative stress markers in serum, sputum or exhaled breath condensate
   a. hydrogen peroxide (H_2O_2 exhalation)
   b. lipid peroxidation (F_2-isoprostanes)
   c. antioxidant enzyme function (post hoc change)
   d. potency (post hoc change)
   e. plasma antioxidant status
   f. plasma fatty acids
2. Inflammation in serum or sputum
   a. inflammatory markers (i.e. IL-6, IL-8, TNF-α, IL-1β)
   b. hyperinflation of chest
3. Nutritional status (e.g. body mass index (BMI) or BMI percentile for children and weight or weight percentile)
4. Pulmonary exacerbations requiring intravenous antibiotic therapy or hospitalisation
5. Adverse events

Since measures of oxidative stress reported were not confined to those anticipated, a post hoc decision was made to include all reported markers of oxidative stress encountered. We categorized oxidative stress outcomes using the classification scheme defined by Dotan (Dotan 2004). Since multiple oxidative stress outcomes exist and within each outcome multiple measures have been identified to quantify the same outcome, oxidative stress was collected as follows.

1. Lipid peroxidation products (F_2-isoprostanes, malondialdehyde (MDA) or thiobarbutic acid reactive substances (TBARS, an unspecific measure of lipid peroxidation), organic hydroperoxides (H_2O_2))
2. Promoters (luminol)
3. Inhibitors (i.e. antioxidant micronutrients and enzymes)
4. Potency (i.e. trolox-equivalent antioxidant capacity (TEAC))
5. Oxidizability (i.e. lag time, propagation)

We also decided to collect data for antioxidant enzymes as measured by erythrocyte glutathione peroxidase (GPX), which is a selenium-dependent enzyme, and superoxide dismutase (SOD).

"Pulmonary exacerbations requiring intravenous antibiotic therapy or hospitalisation" was revised to "days of antibiotic therapy" after data extraction began and data were found to be presented in the latter manner rather than the former.

Search methods for identification of studies

We searched for all relevant published and unpublished studies without restrictions on language, year or publication status.

Electronic searches

Relevant studies were sought from the Cochrane Cystic Fibrosis and Genetic Disorders Group's CF Trials Register using the terms: antioxidants.

The Cystic Fibrosis Trials Register is compiled from electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL) (updated each new issue of the Cochrane Library), weekly searches of MEDLINE, a search of Embase to 1995 and the prospective handsearching of two journals - *Pediatric Pulmonology* and the *Journal of Cystic Fibrosis*. Unpublished work is identified by searching through the abstract books of three major cystic fibrosis conferences: the International Cystic Fibrosis Conference; the European Cystic Fibrosis Conference and the North American Cystic Fibrosis Conference. For full details of all searching activities for the register, please see the relevant sections of the Cystic Fibrosis and Genetic Disorders Group website.

Date of the latest search of the CF Trials Register: 08 January 2019.

We also searched the following databases, trials registries and resources:

1. PubMed (1946 to 31 May 2016);

2. ISRCTN registry (www.isrctn.org, searched 16 July 2018);
3. US National Institutes of Health Ongoing Trials Register Clinicaltrials.gov (www.clinicaltrials.gov, searched 16 July 2018);
4. World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) (apps.who.int/trialsearch, searched 16 July 2018).

See appendices for full search strategies (Appendix 1; Appendix 2).

The previous author team searched the following databases for earlier versions of this review. We were unable to search these for this update because of lack of access.

1. CINAHL Plus EBSCO (Cumulative Index to Nursing and Allied Health Literature; 1937 to December 2007);
2. AMED Ovid (Allied and Complementary Medicine; 1985 to December 2007).

See appendices for full search strategies for previous versions of this review (Appendix 3; Appendix 4).

Searching other resources

We reviewed the reference lists of all included articles and relevant systematic reviews to identify any additional studies. We also contacted investigators of included studies for possible references to previously unidentified RCTs.

Data collection and analysis

Selection of studies

The two authors (OC and JL) assessed studies independently for inclusion into the review. In the case of conflict of opinion between the two authors, they resolved this by discussions until they reached a common agreement. The first stage of screening included systematically screening electronic titles or abstracts (or both) of all studies according to the pre-specified criteria. The authors then reviewed the full-text hard copies, again applying selection criteria.

Data extraction and management

The two authors (OC and JL) extracted data independently for all outcomes of interest using pre-developed extraction forms. In the case of conflict of opinion between the two authors, they resolved this by discussions until they reached a common agreement.

The authors presented different routes of administration as separate comparisons. If one study compared two arms of an antioxidant intervention to control, the authors combined the intervention arms using appropriate statistical methods (*see* Unit of analysis issues).

The concentrations of vitamin E in two of the studies were expressed as mg/100 mL (Harries 1971; Levin 1961) and as µg/mL in two further studies (Visca 2015; Sagel 2018); in this review, the review authors have converted the data from these studies to µmol/L (standard units) by using a converter (http://unitslab.com/node/216).

One study reported separate data for a pediatric cohort and an adult cohort; in order to present these data separately we have generated two study IDs for one single study, one for the adult data (Calabrese 2015a) and one for the pediatric data (Calabrese 2015b).

The authors have reported data at two, three, four, five and six months. If they identify data from other time points for future updates of the review they will consider reporting these too.

Assessment of risk of bias in included studies

The two authors independently assessed the risk of bias of each study following the domain-based evaluation as described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011a). The tool for assessing risk of bias in each included study comprises a judgement and support for the judgement for each entry in a 'Risk of bias' table, where entry addresses a specific feature of the study. The judgement for each entry assesses the risk of bias as low, high or unclear risk, with the last category indicating either lack of information or uncertainty over the potential for bias. In the case of conflict of opinion between the two authors, they resolved this by discussions to lead to a common agreement.

They assessed the domains listed below.

1. Randomisation
2. Concealment of allocation
3. Blinding (of participants, personnel and outcome assessors)
4. Incomplete outcome data (whether investigators used an intention-to-treat analysis)
5. Selective outcome reporting
6. Other potential threats to validity

Measures of treatment effect

For binary outcomes (hyperinflation of the chest, number participants with pulmonary exacerbations and adverse events), the review authors planned to report relative risks (RR) and 95% confidence intervals (CIs). When possible, they reported the proportion of participants reporting adverse events for each treatment arm. As they expected adverse events to be rare, they analysed these outcomes using the Peto odds ratio (OR) statistic and 95% CIs.

The review authors recorded continuous outcomes (lung function, QoL, markers of oxidative stress, inflammatory markers and markers of nutritional status) as either mean relative changes from baseline or mean end-point values and standard deviations (SD). Where studies reported standard errors (SE), the review authors converted these to SDs. They calculated the mean difference (MD) and 95% CI for most outcome measures except for outcomes of oxidative stress which combined multiple measures and for QoL in the comparison of oral antioxidants versus control (where studies used different questionnaires) for which they used standardized mean differences (SMDs) and 95% CI.

Unit of analysis issues

Cross-over studies

If the review authors had been able to include cross-over studies with sufficient data, they planned to analyse these by paired t-test for continuous data, as long as there was no evidence of carry-over or period effect (Elbourne 2002). Where papers reported cross-over study data insufficiently, i.e. so that only first-period data were available, the review authors treated data from the first period as a parallel study (Elbourne 2002).

Studies with multiple treatment arms

For studies reporting multiple intervention and placebo groups, the review authors combined all relevant intervention groups and placebo groups, each to be analysed as a single group as recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* to avoid a unit of analysis error (Higgins 2011b).

Dealing with missing data

Review authors made up to two attempts to contact each of the investigators for each study from which information was missing. If the investigators did not respond, the review authors left out incomplete data.

The review authors received additional data from the investigators of six studies which they used in the analysis (Calabrese 2015a; Calabrese 2015b; Conrad 2015; Dauletbaev 2009; Griese 2013; Keljo 2000; Visca 2015).

Assessment of heterogeneity

Review authors planned to measure the inconsistency of study results using the Chi^2 test and the I^2 heterogeneity statistic to determine if variation in outcomes across studies was due study heterogeneity rather than chance (Higgins 2003). This Chi^2 test assesses whether observed differences in results are compatible with chance alone. A low P value (or a large Chi^2 statistic relative to its degree of freedom) provides evidence of heterogeneity of intervention effects (variation in effect estimates beyond chance). A P value of 0.10, rather than the conventional level of 0.05, is used to determine statistical significance.

The I^2 statistic, as defined by Higgins (Higgins 2011a), measures heterogeneity as a percentage where a value:

- 0% to 40%: might not be important;
- 30% to 60%: may represent moderate heterogeneity;
- 50% to 90%: may represent substantial heterogeneity;
- 75% to 100%: considerable heterogeneity.

The importance of the observed value of I^2 depends on (i) magnitude and direction of effects and (ii) strength of evidence for heterogeneity (e.g. P value from the Chi^2 test, or a confidence interval for I^2).

Assessment of reporting biases

Using the method by Light, if the review authors had included a sufficient number of studies (at least 10, by convention) combined in a single meta-analysis, they planned to assess publication bias using a funnel plot (Light 1994). A funnel plot is a graph that plots treatment effect for each study against a measure of precision (i.e. 1/standard error (SE)).

The review authors present information regarding selective reporting of outcomes within individual studies in the risk of bias assessment (Risk of bias in included studies).

Data synthesis

The main comparisons were between antioxidant supplementation and control (standard of care, other therapy, no treatment). The review authors have presented a forest plot for each outcome for which data are available. Where they have included more than one study for a single subgroup, they have pooled data into a single effect estimate. Since each antioxidant works by a different mechanism of action, they analysed each micronutrient or unique combination of micronutrients as a separate subgroup, as per the first originally planned subgroup analysis to explore methodological heterogeneity.

The review authors intended to use a fixed-effect model for all analyses with a low degree of heterogeneity (I^2 less than 40%). They later decided to employ a random-effects model for all analyses, since there were known differences (i.e. doses, duration and solubility of supplement) and unknown differences between studies that may potentially influence the size of the treatment effect.

The review authors analysed all studies using the Review Manager software (RevMan 2014).

Subgroup analysis and investigation of heterogeneity

Where the review authors included at least 10 studies per outcome (Deeks 2011), they planned the following a priori subgroup analyses to investigate both clinical and methodological heterogeneity.

Clinical heterogeneity

Planned clinical subgroups were:

- age: pediatric (up to 18 years) versus adult (over 18 years);
- disease severity as measured by FEV_1 (70% to 80% considered mild; 60% to 70% moderate; 50% to 60% moderately severe; 34% to 50% severe; and less than 34% very severe as defined by American Thoracic Society guidelines (Pellegrino 2005)).

Methodological heterogeneity

Planned methodological subgroups were:

- combined antioxidant supplementation and single antioxidant supplementation (i.e. each single micronutrient or combination thereof are listed separately);
- antioxidant(s) alone versus antioxidant(s) alongside concurrent treatment;
- timing of intervention: antioxidant(s) as prophylactic or therapeutic treatment.

Post hoc, the review authors decided that, regardless of the number of studies per outcome, individual supplements or unique combinations thereof should not be combined in a single meta-analysis as it would not be appropriate due to the aforementioned differences between micronutrients. Therefore, the review authors presented results for different supplement interventions separately.

Sensitivity analysis

While the protocol for this review indicated that the review authors would base sensitivity analysis on only randomisation, allocation concealment, blinding, and intention-to-treat versus per-protocol analysis, they later decided to evaluate risk of bias using the newly introduced risk of bias tool, therefore altering planned sensitivity analyses.

The review authors planned sensitivity analyses to evaluate treatment effect by excluding studies with an overall high risk of bias.

Summary of findings tables

In a post hoc change from protocol, the review authors have presented two summary of findings tables, one for oral antioxidants versus control and one for inhaled antioxidants versus control (Summary of findings for the main comparison; Summary of findings 2).

The following outcomes were reported in all tables (chosen based on relevance to clinicians and consumers):

- FEV_1 % predicted at three months (change from baseline);
- FEV_1 % predicted at six months (change from baseline);
- QoL (change from baseline) at six months;
- BMI (change from baseline) at six months;
- mean time to next pulmonary exacerbation (at six months);
- adverse effects.

We determined the quality of the evidence using the GRADE approach; and downgraded evidence in the presence of a high risk of bias in at least one study, indirectness of the evidence, unexplained heterogeneity or inconsistency, imprecision of results, high probability of publication bias. We downgraded evidence by one level if they considered the limitation to be serious and by two levels if very serious.

RESULTS

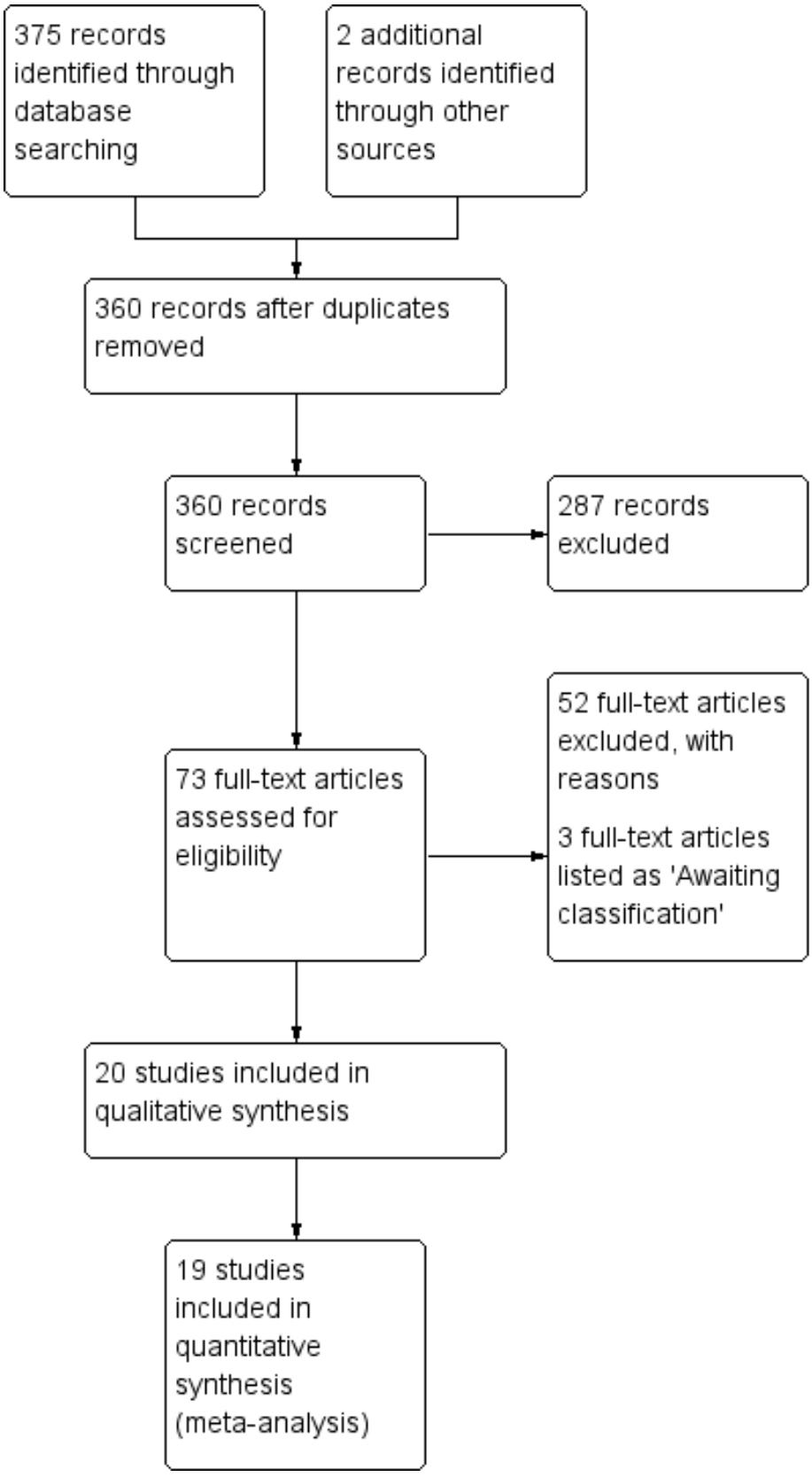
Description of studies

Results of the search

Out of 360 unique studies yielded from the search strategy, 73 remained after title and abstract screening. After full text screening, 20 studies met the inclusion criteria (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Conrad 2015; Dauletbaev 2009; Götz 1980; Griese 2013; Harries 1971; Homnick 1995b; Howatt 1966; Keljo 2000; Levin 1961; Mitchell 1982; Portal 1995a; Ratjen 1985; Renner 2001; Stafanger 1988; Stafanger 1989; Visca 2015; Wood 2003; Sagel 2018). Three studies remain listed under 'Characteristics of studies awaiting classification' as they are only currently available in abstract format and, if included, may compromise the validity of results due to unavailability of a complete set of data (Tirouvanziam 2005; Tirouvanziam 2006; Wong 1988). A total of 52 studies were excluded with reasons as detailed below (Excluded studies).

The flow of studies through the screening process of the review is shown in the figures (Figure 2); this process uses the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Moher 2009). During full-text screening, three study reports were translated but did not meet final inclusion criteria.

Figure 2. Study flow diagram.



We have received additional data from the authors of five studies (six data sets) (Calabrese 2015a; Calabrese 2015b; Conrad 2015; Dauletbaev 2009; Griese 2013; Keljo 2000; Visca 2015); these data have been used in the analyses.

Included studies

Six of the included studies were represented by single articles (Dauletbaev 2009; Götz 1980; Howatt 1966; Keljo 2000; Mitchell 1982; Wood 2003) and one report represented two studies, one of which was included and one excluded (Homnick 1995a; Homnick 1995b). Several of the included studies had multiple papers linked to them. Two reports (an article and a conference abstract) represented each of the Griese, the Harries, the Ratjen and both Stafanger studies (Griese 2013; Harries 1971; Ratjen 1985; Stafanger 1988; Stafanger 1989); two reports represented the Portal study (Portal 1995a) and there were also two reports (an article and a letter) for the Levin study (Levin 1961). There are three reports on the Visca study, two conference abstracts (one poster) and a published paper (Visca 2015). There are three abstracts and one full report representing each of the Bishop, the Calabrese and the Conrad studies (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Conrad 2015). There were four reports for the Sagel study, a record on ClinicalTrials.gov, two abstracts and a full paper (Sagel 2018). There were three reports and four abstracts representing the Renner study (Renner 2001).

Oral antioxidant supplements were given in 16 studies (Conrad 2015; Dauletbaev 2009; Götz 1980; Harries 1971; Homnick 1995b; Keljo 2000; Levin 1961; Mitchell 1982; Portal 1995a; Ratjen 1985; Renner 2001; Sagel 2018; Stafanger 1988; Stafanger 1989; Visca 2015; Wood 2003) and four used nebulised supplements (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013; Howatt 1966). We present the characteristics of these studies separately below.

Study characteristics

Oral supplementation

Five studies were conducted in the USA (Conrad 2015; Homnick 1995b; Keljo 2000; Levin 1961; Sagel 2018), one in Australia (Wood 2003), one in New Zealand (Mitchell 1982); the remaining nine studies were conducted in Europe (one in France (Portal 1995a), one in Italy (Visca 2015), one in Great Britain (Harries 1971), two in Austria (Götz 1980; Renner 2001), two in Germany (Dauletbaev 2009; Ratjen 1985) and two in Denmark (Stafanger 1988; Stafanger 1989)). One study did not contain any information regarding sequence generation or allocation concealment has been interpreted as a controlled clinical study (Homnick 1995b); the remaining 15 studies were RCTs.

There are 11 studies of parallel design (Conrad 2015; Dauletbaev 2009; Harries 1971; Homnick 1995b; Keljo 2000; Levin 1961; Ratjen 1985; Renner 2001; Sagel 2018; Visca 2015; Wood 2003) and the remaining five are of cross-over design (Götz 1980; Mitchell 1982; Portal 1995a; Stafanger 1988; Stafanger 1989). In the Portal study each arm lasted five months with a two-month washout period between treatment periods (Portal 1995a). A two-week washout period between the three-month treatment periods was reported in the Mitchell study (Mitchell 1982). In the remaining three studies, no washout period between the periods of placebo and treatment administration were reported (Götz 1980; Stafanger 1988; Stafanger 1989).

Time points for reporting data in the included studies ranged from one month to 12 months (see Table 1).

The source of funding was reported in 12 out of the 16 studies of oral supplements; of these, one author (DPRM) on the Harries study was supported by Roche Products Ltd (Harries 1971), Stafanger was supported by ASTRA A/S Copenhagen (Stafanger 1988; Stafanger 1989) and Dauletbaev by Hexal AG, Germany (Dauletbaev 2009). In none of the remaining eight studies reporting funding sources did authors receive funding from industry (Conrad 2015; Griese 2013; Homnick 1995b; Keljo 2000; Portal 1995a; Sagel 2018; Visca 2015; Wood 2003). However, it should also be noted that four of the co-authors on the Conrad study are listed as inventors on a provisional patent application covering NAC as a therapeutic agent for CF (Conrad 2015).

Inhaled supplementation

Two studies were conducted in the USA (Bishop 2005; Howatt 1966) and two were conducted in Europe (one in Germany (Griese 2013) and one in Italy (Calabrese 2015a; Calabrese 2015b)). All four studies were RCTs (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013; Howatt 1966). Three studies were of parallel design (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013) and one was of cross-over design (Howatt 1966). The cross over study did not report any washout period between treatments.

Time points for reporting data in the included studies ranged from one month to 12 months (see Table 2).

The source of funding was reported in two out of the four included studies of inhaled supplements, but funding was not from industry (Bishop 2005; Calabrese 2015a; Calabrese 2015b).

Participants

Oral supplementation

The 16 studies of oral supplementation represent 639 participants and sample size ranged from 20 participants (Homnick 1995b; Mitchell 1982) to 70 participants (Conrad 2015). Four studies reported the use of power calculations in determining sample size (Conrad 2015; Keljo 2000; Sagel 2018; Visca 2015), and three of these related these calculations to their primary outcome (Conrad 2015; Keljo 2000; Sagel 2018). The age of participants was not consistently reported in all studies, but the minimum reported age for inclusion was six months (Harries 1971) and maximum was 59 years (Conrad 2015). Of the 16 included studies of oral antioxidant supplementation, one did not report the age of participants (Homnick 1995b); six included just children, but with a large range of ages from 18 months to 16 years (Götz 1980; Harries 1971; Levin 1961; Mitchell 1982; Visca 2015; Wood 2003); and eight included a mixture of children and adults (Conrad 2015; Keljo 2000; Portal 1995a; Renner 2001; Ratjen 1985; Stafanger 1988; Stafanger 1989; Sagel 2018) and one only adults (Dauletbaev 2009).

The gender of the participants was reported by 14 studies (Conrad 2015; Dauletbaev 2009; Götz 1980; Keljo 2000; Levin 1961; Mitchell 1982; Portal 1995a; Ratjen 1985; Renner 2001; Sagel 2018; Stafanger 1988; Stafanger 1989; Wood 2003; Visca 2015). Details of gender split were not reported by the two remaining studies (Harries 1971; Homnick 1995b). There were approximately equal numbers of males and females in 10 studies (Conrad 2015; Götz 1980; Keljo 2000; Mitchell 1982; Portal 1995a; Ratjen 1985; Sagel 2018; Stafanger 1988; Stafanger 1989; Visca 2015). Although in the Visca

study there were more females (58%) than males in the treatment group, but more males (57%) than females in the placebo group (Visca 2015); in the Conrad study, there were 56% males in the treatment group and 44% males in the control group (Conrad 2015). In two studies there were more males than females; in the Levin study overall there were 57% male (68% in the placebo group but only 45% in the treatment group) (Levin 1961) and in the Dauletbaev study, there were nine out of the 11 participants in the 700 mg daily NAC group were male and seven out of 10 participants in the 2800 mg daily NAC were male (Dauletbaev 2009). In two studies there were more females than males overall; Wood reported 45% of participants overall were male, 59% males in the treatment group but only 33% in the placebo group (Wood 2003). There were also significantly fewer males (25%) than females reported in the Renner study (Renner 2001).

Given the small number of studies included in the review, we were not able to split data by clinical subgroups.

Inhaled supplementation

The four studies of inhaled supplementation included in this review represent 285 participants and sample sizes ranged from eight (Howatt 1966) to 153 participants (Griese 2013). Two studies reported the use of power calculations in determining sample size related to their primary outcome (Calabrese 2015a; Calabrese 2015b; Griese 2013). For the four studies of nebulised supplements, one included people with CF with a mean age of 23 years (Griese 2013), one included a pediatric group with a mean (SD) age of 12.8 (3.1) years and an adult group with a mean (SD) age of 27.66 (8.25) years (Calabrese 2015a; Calabrese 2015b), one included people with CF aged 6 to 19 years with a mean age of 13 years (Bishop 2005) and the remaining study included people with CF ranging in age from six years to 23 years (Howatt 1966).

All four studies reported the gender of the participants (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013; Howatt 1966). There were approximately equal numbers of males and females in three studies (Calabrese 2015a; Calabrese 2015b; Griese 2013; Howatt 1966), but there were more males than females in the Bishop study: 67% in the treatment group and 60% in the placebo group (Bishop 2005).

Given the small number of studies included in the review, we were not able to split data by clinical subgroups.

Interventions

Oral antioxidant supplements were given in 16 studies (Conrad 2015; Dauletbaev 2009; Götz 1980; Harries 1971; Homnick 1995b; Keljo 2000; Levin 1961; Mitchell 1982; Portal 1995a; Ratjen 1985; Renner 2001; Sagel 2018; Stafanger 1988; Stafanger 1989; Visca 2015; Wood 2003) and four used nebulised supplements (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013; Howatt 1966). There were not enough data to examine other planned methodological subgroups. Data were grouped according to combined and single supplementation such that each unique micronutrient or combination thereof were presented separately. Since at least 10 studies are thought to be necessary for meaningful subgroup analysis (Deeks 2011), the subgroup analyses presented are meant to be exploratory.

Oral supplementation

Participants in all studies received standard pancreatic enzymes and vitamin supplements in addition to the study interventions.

Three studies evaluated supplementation with vitamin E (α-tocopherol) (Harries 1971; Keljo 2000; Levin 1961). Harries compared supplementation with vitamin E (10 mg/kg/day D,L-α-tocopheryl acetate) in a single daily dose to control group without vitamin E supplement; both a fat-soluble and a water-miscible preparation were assessed (Harries 1971). The second study compared supplementation with vitamin E (naturally occurring RRR-α-tocopherol) in tablet form to placebo; doses of the supplement were determined according to weight - 600 IU/day for participants under 20 kg and 1200 IU/day for participants who weighed over 20 kg (1 IU is the biological equivalent of 0.45 mg of D,L-α-tocopheryl acetate) (Keljo 2000). Levin compared supplementation with vitamin E in a dose of 10 mg/kg/day of D,L-α-tocopheryl acetate in a water-miscible dispersion divided in two or three doses to placebo (Levin 1961).

Two studies examined β-carotene supplementation (Homnick 1995b; Renner 2001). Homnick reports on a comparison of participants in the β-carotene group who received 30 mg β-carotene twice a day (60 mg/day) to a control group (Homnick 1995b). The β-carotene dose was increased individually and periodically during the study in an attempt to obtain plasma concentrations of 0.37 to 0.74 μM/L believed to be consistent with baseline concentrations in normal people. Eight participants in the control and five in the β-carotene group finished the study, but there are no data reported from the control group (Homnick 1995b). In the Renner study, investigators compared a weight-dependent dose of β-carotene (1 mg/kg of body weight/day up to a maximum of 50 mg/day) to placebo for three months, after which point the β-carotene was supplemented in a standard, non-weight-dependent dose (10 mg/day) for all participants for another three months (Renner 2001). Since the average weight-dependent dose during the first part of the study was not reported, measurements at this time point were not meaningful and only endpoint data (i.e. change from baseline to six months) were included for meta-analysis.

A further study examined selenium supplementation in a cross-over study (Portal 1995a). The investigators examined a 2.8 mg/kg of body weight/day dose of selenium compared to placebo (Portal 1995a).

One study evaluated a combination of 200 mg vitamin E, 300 mg vitamin C, 25 mg β-carotene, 90 μg selenium and 500 μg vitamin A compared to routine vitamin treatment (10 mg vitamin E and 500 μg of vitamin A) (Wood 2003). The vitamin E supplement was administered as RRR-α-tocopherol.

One study examined a mixed supplementation (AquADEKs-2) containing standard amounts of fat-soluble vitamins (A, D, E, K) that are contained in typical CF multivitamin supplements plus several antioxidants including β-carotene, mixed tocopherols (different forms of vitamin E), co-enzyme Q10 (CoQ10), mixed carotenoids (lutein, lycopene and zeaxanthin), and the minerals zinc and selenium (Sagel 2018). This treatment was compared to a control multivitamin containing standard amounts of vitamins A, B, D, E, and K for CF but without added antioxidants.

The remaining eight studies assessed oral supplementation with L-glutathione or NAC as a source of GSH (Conrad 2015; Dauletbaev

2009; Götz 1980; Mitchell 1982; Ratjen 1985; Stafanger 1988; Stafanger 1989; Visca 2015). Just one study evaluated oral supplementation with L-glutathione (65 mg/kg/day) divided into three doses per day compared to placebo (calcium citrate 65 mg/kg/day) (Visca 2015). Seven studies evaluated different dose regimens of NAC compared to placebo. One study administered 900 mg NAC as effervescent tablets three times daily (2700 mg/day) for 24 weeks compared to placebo (Conrad 2015). Four studies used doses of oral NAC of 200 mg three times daily (600 mg/day) (Mitchell 1982; Ratjen 1985; Stafanger 1988; Stafanger 1989). In both Stafanger studies the dose was increased to 400 mg three times daily (1200 mg/day) if the body weight of the participants was over 30 kg. In the Götz study NAC was given twice daily with an average dosage of 9.5 mg/kg and compared to placebo (Götz 1980). The remaining study compared NAC 700 mg/day (one tablet with NAC 700 mg and three placebo tablets) to NAC 2800 mg/day (four tablets each containing NAC 700 mg) in two groups of participants; additional data from this study have been obtained after contacting the author (Dauletbaev 2009).

Inhaled supplementation

Four studies reported on the supplementation with nebulised GSH compared to placebo (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013; Howatt 1966). In the Bishop study, participants inhaled buffered GSH 66 mg/kg distributed across four inhalation sessions per day (spaced three to four hours apart) for eight weeks, full dose in the last six weeks (Bishop 2005). In a second study, the participants inhaled GSH twice daily at a dose of 10 mg/kg body weight (maximum dose 600 mg) (Calabrese 2015a; Calabrese 2015b). Likewise, in the Griese study, GSH was inhaled twice daily but at a dose of 646 mg every 12 hours via an eFlow nebulizer (Griese 2013). In the fourth study, Howatt compared 5 mL of NAC at two different concentrations, 20% and 2% (placebo), three times daily (Howatt 1966).

Outcomes

Outcomes for the different interventions are reported and analysed separately.

Oral supplementation

A total of 11 studies reported on lung function, a primary outcome of this review (Conrad 2015; Dauletbaev 2009; Götz 1980; Mitchell 1982; Ratjen 1985; Renner 2001; Sagel 2018; Stafanger 1988; Stafanger 1989; Visca 2015; Wood 2003). Of these, one study reported peak expiratory flow values, a parameter not included in the analysis (Mitchell 1982). The remaining 10 studies reported FEV_1, but data for the change from baseline values, as stated in our analysis plan, were only available from seven studies (Conrad 2015; Dauletbaev 2009; Ratjen 1985; Sagel 2018; Stafanger 1989; Wood 2003;Renner 2001); three studies reported narrative information for FEV_1 (Götz 1980; Mitchell 1982; Stafanger 1988). Six studies additionally reported FVC as % predicted (Conrad 2015; Dauletbaev 2009; Stafanger 1988; Stafanger 1989; Visca 2015; Wood 2003). Visca reported the spirometry data only as poster results and these data were not included in the published article (Visca 2015).

Two studies reported QoL using a validated measures - CF Quality of Life Questionnaire respiratory domain scale (CFQ-R) (Conrad 2015) and quality of well-being (QoWB) (Wood 2003).

For markers of oxidative stress, four studies reported lipid peroxidation measures: one reported F_2-isoprostanes in plasma (Wood 2003); one reported urine 8-iso-PGF2 (Sagel 2018) one reported organic peroxides measurements (Portal 1995a), two studies reported thiobarbituric acid reactive substances (TBARS) (Portal 1995a; Sagel 2018) and one reported malondialdehyde (MDA) levels measured by HPLC (Renner 2001). However, TBAR levels in (mcM) units reported by the Sagel study were not compatible with the other reported data and were not used in the analysis (Sagel 2018). Two studies reported glutathione peroxidase (GPX) function (Portal 1995a; Wood 2003) and one reported superoxide dismutase (SOD) activity (Wood 2003). One study reported oxidative stress potential by trolox-equivalent antioxidant capacity (TEAC) (Renner 2001) and two reported total antioxidant capacity (Renner 2001; Sagel 2018), though measured by different methods; either by a photometric method according to Rice-Evans and Miller (Renner 2001) or by copper reduction-colorimetric assay (CRE) (Sagel 2018). Conrad reported sputum neutrophil elastase activity, sputum neutrophil count, sputum and plasma IL-8 (Conrad 2015). Dauletbaev reported sputum neutrophil counts, TNF-α and IL-8 in induced sputum (Dauletbaev 2009) and a further study reported on levels of sputum myeloperoxidase, as a measure of neutrophil activity (Sagel 2018).

Five studies reported changes in the plasma levels of α-tocopherol (vitamin E) (Harries 1971; Keljo 2000; Levin 1961; Sagel 2018; Visca 2015). However, in the Visca study participants received oral supplementation with GSH and therefore the effect on the level of vitamin E is indirect (Visca 2015). As stated above, the concentrations of vitamin E in the studies were expressed as either mg/100 mL (Harries 1971; Levin 1961) or as µg/mL (Sagel 2018; Visca 2015) and have been converted to µmol/L by the review authors. One study of NAC supplementation measured the GSH in whole blood (Conrad 2015), while another study of NAC supplementation measured extracellular levels of GSH in induced sputum (Dauletbaev 2009). One study measured the plasma fatty acid status of 17 plasma fatty acids; since we did not pre-specify which to analyse, only data for total plasma fatty acid status were included in our analysis (Wood 2003). One study reported plasmid total lipids as a correction factor for α-tocopherol levels and could not be included in the analysis (Sagel 2018).

Four studies measured β-carotene antioxidant status (Homnick 1995b; Portal 1995a; Renner 2001; Sagel 2018). However, one of these did not completely report endpoints for the control group; as such, we did not have complete data to enter into a meta-analysis or report in the text narratively (Homnick 1995b).

Four studies measured BMI (Conrad 2015; Renner 2001; Visca 2015;Sagel 2018); one of these reported both percentile and z scores (Visca 2015). Two studies did not provide complete outcome data (Renner 2001; Sagel 2018). Five studies reported weight (Conrad 2015; Levin 1961; Mitchell 1982; Visca 2015,Sagel 2018); three studies measured this outcome in kg (Conrad 2015; Levin 1961; Mitchell 1982), one reported both percentile and z scores (Visca 2015) and one reported z scores (Sagel 2018).

Three studies reported the number of days of antibiotic therapy (Mitchell 1982; Renner 2001; Wood 2003). Two studies reported the number of pulmonary exacerbations; one also reported the number of hospitalizations (Conrad 2015), while the second

additionally reported the time to first pulmonary exacerbation (Sagel 2018).

Data on death during the studies or adverse events were reported in eight studies (Conrad 2015; Dauletbaev 2009; Keljo 2000; Levin 1961; Portal 1995a; Renner 2001; Sagel 2018; Visca 2015). Conrad particularly assessed the development of pulmonary hypertension in light of a study by Palmer which reported that chronic, systemic administration of either NAC or S-nitroso-acetylcysteine caused hypoxia-mimetic pulmonary hypertension in mice (Palmer 2007).

Keljo measured cytokines as the primary outcome measure of the study (Keljo 2000). Sagel measured sputum myeloperoxidase as the primary outcome (Sagel 2018).

Inhaled supplementation

All the studies assessing GSH or NAC inhalation reported on lung function, the primary outcome of this review, but used a range of measures (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013; Howatt 1966). Bishop reported the MDs (post-baseline) between GSH and placebo groups for FEV_1, FVC and FEF_{25-75} in % predicted compared to normal values and peak flow measures (Bishop 2005). The second study reported MDs (post-baseline) for FEV_1 and FEF_{25-75} in both % predicted and L; the study authors were contacted and have provided values for FVC in L and % predicted (Calabrese 2015a; Calabrese 2015b). The Griese study presents data on changes in the absolute FEV_1; after contacting the author, data for mean differences (post-baseline) in FEV_1, FVC and FEF_{25-75} in % predicted have been also obtained (Griese 2013). The Howatt study reported only qualitative data, such as "improve" or "worse" compared to baseline (Howatt 1966).

Three of the four studies reported on QoL, but used different methods impeding the meta-analysis (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013); Bishop used self-reported parameters, Calabrese used version 2.0 of the Italian validated questionnaire (CFQoL) (Monti 2008) and Griese used a validated measurement tool (Wenninger 2003).

One study reported markers of oxidative stress, measurements of GSH and its metabolites in both sputum and blood (Griese 2013). Both reduced GSH and reduced forms of its metabolites (named free GSH or free forms) and the sum of reduced and oxidized GSH and that of its metabolites (named total GSH or total forms) were measured. The intracellular levels of GSH in neutrophils from sputum and blood were also measured in a small subgroup of participants. Lipid mediators (isoprostanes) and protein carbonyls were reported in sputum (Griese 2013). A second study reported H_2O_2 in exhaled breath condensate and serum (Calabrese 2015a; Calabrese 2015b). Griese also reported cytokines (IL-10, IL-8) in the sputum in a subgroup of participants from the intervention group compared to placebo (Griese 2013).

Three studies reported changes in nutritional status (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013). Bishop reported the average difference in BMI between baseline and after two months (Bishop 2005); while Calabrese reported the mean BMI at baseline and after 12 months in each group of adults and children (Calabrese 2015a; Calabrese 2015b); Griese measured weight (Griese 2013).

Two studies reported the number of exacerbations per participant (Calabrese 2015a; Calabrese 2015b; Griese 2013).

Three studies reported adverse events (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013).

Excluded studies

Upon title and abstract screening 287 studies were excluded and a further 52 were excluded after full-text screening (*see* Characteristics of excluded studies). We excluded 12 studies described as controlled studies from this review (Cobanoglu 2002; Congden 1981; Farrell 1977; Knopfle 1975; Lancellotti 1996; Lepage 1996; Madarasi 2000; Portal 1995b; Underwood 1972b; Winklhofer-Roob 1995; Winklhofer-Roob 1996c; Winklhofer-Roob 1997a). In a further four studies, the antioxidant intervention was compared to an active control arm, therefore not meeting the pre-specified selection criteria for the review (Nasr 1993; Papas 2007; Peters 1996; Winklhofer-Roob 1996b); in one study, a micronutrient mix was compared to placebo; however, the intervention contained a mixture of micronutrients in addition to those being studied and the sole effects of those of interest could not be obtained (Oudshoorn 2007); seven studies did not include any of the interventions under consideration (Abdulhamid 2008; Best 2004; Khorasani 2009; Mischler 1991; Powell 2010; Sharma 2016; Wojewodka 2015). We also excluded 12 prospective cohort studies (Bines 2005; Ekvall 1978; Kauf 1995; Kawchak 1999; Kelleher 1987; Munck 2010; Rawal 1974; Rettammel 1995; Richard 1990; Sokol 1989; Sung 1980; Wood 2002), seven review articles (Anonymous 1975; Beddoes 1981; Goodchild 1986; Oermann 2001; van der Vliet 1997; Winklhofer-Roob 2003; Zoirova 1983), three concerned letters (Winklhofer-Roob 1996a; Winklhofer-Roob 1997b; Winklhofer-Roob 1997c), two studies reporting on single-dose administration for tolerance investigations (Homnick 1995a; Jacquemin 2009), two case-reports (Hoogenraad 1989; Hubbard 1980), one retrospective cohort study (Underwood 1972a) and one study in people with chronic pancreatitis (Uden 1990).

Out of the excluded studies, two were represented by three separate reports (Winklhofer-Roob 1996c; Wojewodka 2015) and two were represented by a report and an abstract (Abdulhamid 2008; Winklhofer-Roob 1996b). The remaining studies were each represented by a single report.

Studies awaiting classification

A total of three studies are listed as 'Awaiting classification' as to date they are only available in abstract form and their inclusion may compromise the validity of results due to unavailability of a complete set of data (Tirouvanziam 2005; Tirouvanziam 2006; Wong 1988).

Two studies employed NAC as the intervention (Tirouvanziam 2005; Tirouvanziam 2006). The first was a phase I parallel study which randomised 18 participants with CF to one of three different doses (1.8 g/day, 2.4 g/day and 3.0 g/day) given three times daily for four weeks (Tirouvanziam 2005). This study measured mainly adverse effects, QoL and GSH levels in blood, cells and sputum. Following on from this study, the same team undertook a phase 2 parallel, placebo-controlled study of NAC at a dose of 2.7 g/day given over three doses for a duration of 12 weeks (Tirouvanziam 2006). This initial 12-week phase randomised 21 participants with CF and was followed by a further 12-week open-label drug-only phase, the results of which will not be eligible for inclusion in this

review. However, if we are able to obtain results from the first randomised 12-week phase we will included these data. Outcomes assessed included IIve neutrophil count in sputum, pulmonary function tests, adverse events, CF QoL, complete blood count, serum chemistries and intracellular GSH.

The third study listed as awaiting classification pending further information is of fat-soluble vitamin E (Wong 1988). The study describes a parallel study with three arms, but the method of randomisation is not described. The study recruited 30 people with CF admitted to hospital with a pulmonary exacerbation, hence the duration of treatment is between 10 and 14 days. The three treatment arms are oral fat-soluble vitamin E (10 mg/kg/day), or oral water-miscible vitamin E (10 mg/kg/day) or no supplement. The outcomes measured were serum vitamin E levels and three-day fecal fat excretion.

Risk of bias in included studies

As can be seen from the risk of bias summaries none of the 20 included studies was free of bias and when each of the domains are considered across studies, none of the domains were apparently free of bias (Figure 3; Figure 4). Of those studies that had assessable (i.e. not unclear) domains (green and red dots), there were 18 instances of studies being judged to have a high risk of bias and 42 instances of a low risk of bias assessment. Most studies failed to adequately describe allocation concealment and blinding, resulting in an unclear risk of bias with respect to these domains (yellow dots). Each domain is individually described below.

Figure 3. Risk of bias graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.

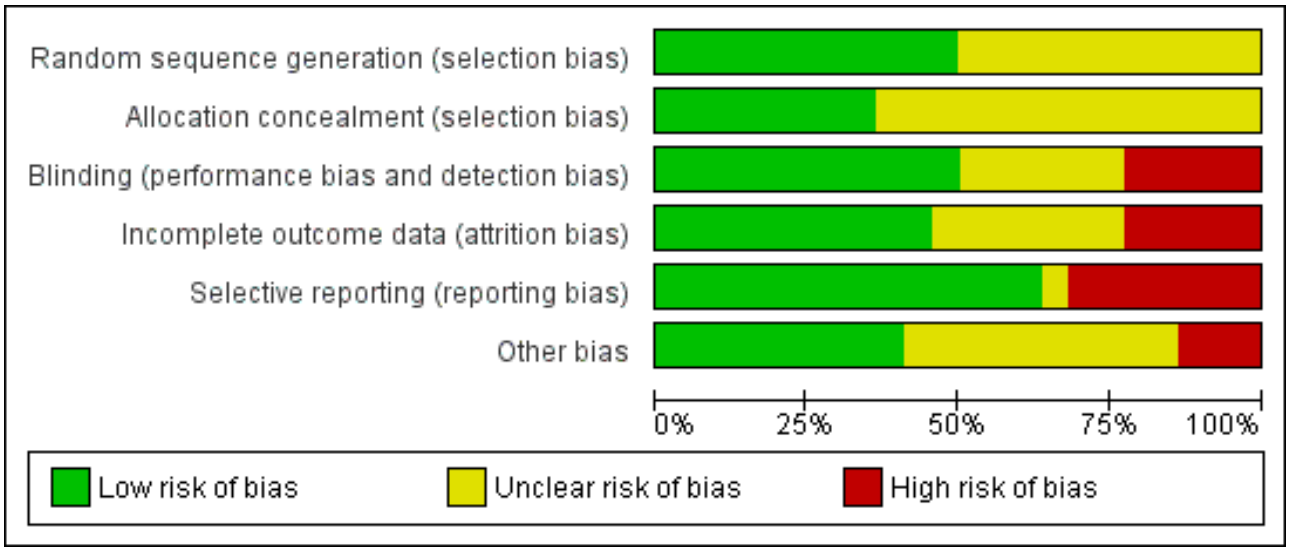


Figure 4. Risk of bias summary: review authors' judgements about each methodological domain for each included study.

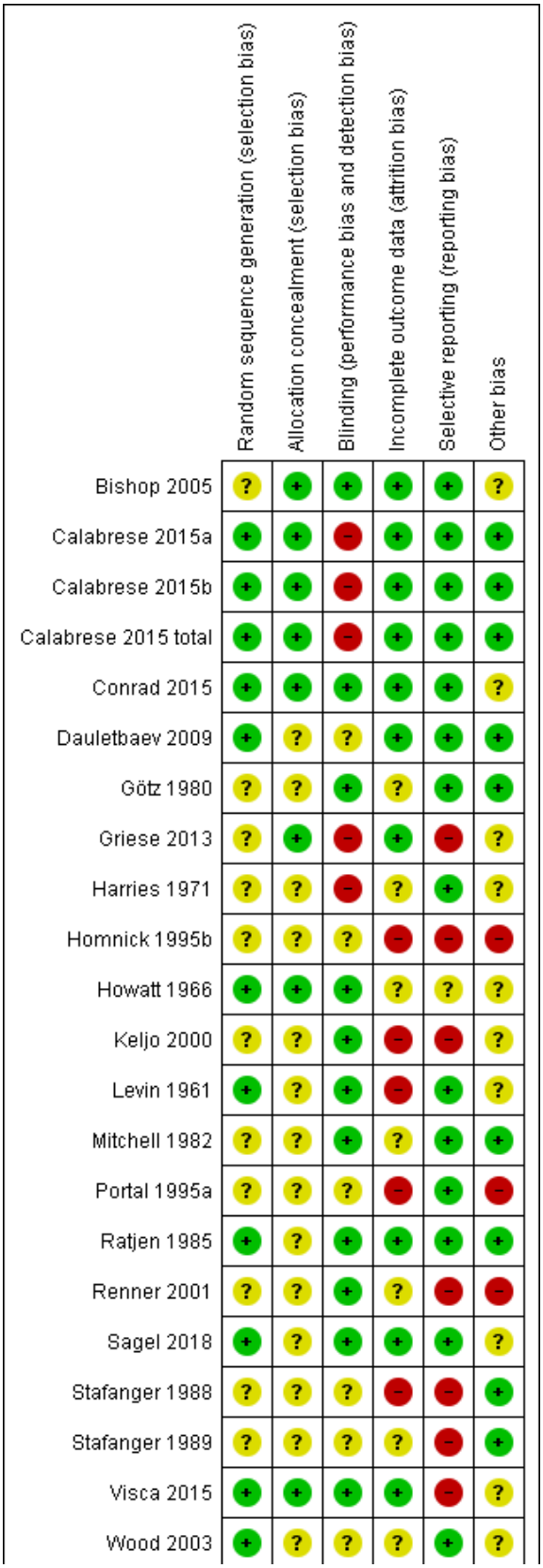


Figure 4. (Continued)

Allocation

Sequence generation

Eight studies adequately described sequence generation and were judged to have a low risk of bias (Calabrese 2015a; Calabrese 2015b; Conrad 2015; Dauletbaev 2009; Howatt 1966; Levin 1961; Ratjen 1985; Visca 2015; Wood 2003). Three studies stated that computer-generated randomisation lists were used to assign participants groups (Calabrese 2015a; Calabrese 2015b; Ratjen 1985; Wood 2003). Conrad used a secure randomisation system by PPD, Inc. to generate assignments which were then distributed to each participating centre (Conrad 2015). Dautlebaev also used a computerized system (Random 1.0 software) to generate the randomisation sequence in a ratio of 1:1 for the different NAC doses (Dauletbaev 2009). Visca used a random number generator to randomly assigned participants to treatment or placebo as specified in the protocol (supplementary data available online) (Visca 2015). In the Howatt study the order of treatment arms was determined by making two slips of paper for each of the six possible combinations and having the participant draw its schedule from an envelope (Howatt 1966). In the Levin study, cards labelled 1 or 2 were individually placed in sealed envelopes in groups of four, two for each mixture number. Envelopes were divided into three groups, according to the age of the participants: less than 5 years, between 5 and 10 years and 10 years or older (Levin 1961). In Sagel study (Sagel 2018), participants were randomised 1:1 to receive either the antioxidant-enriched multivitamin ("treated" group) or continue on control multivitamin ("control" group). An adaptive randomisation algorithm was employed based on stratification factors for: age (10 to 17 years, over 18 years), FEV_1 % predicted (40% to 70%, over 70% and up to 100%), chronic use of inhaled antibiotics, and chronic use of azithromycin.

We judged there to be an unclear risk of bias for the remaining 11 studies (Bishop 2005; Götz 1980; Griese 2013; Harries 1971; Homnick 1995b; Keljo 2000; Mitchell 1982; Portal 1995a; Renner 2001; Stafanger 1988; Stafanger 1989). In the Bishop study, it is stated that participants were first paired by age and sex, and then each member of the pair was randomly assigned to the treatment or placebo group, but the actual method of randomisation is not described (Bishop 2005). Similarly, Griese randomised participants by central telephone block randomisation at 1:1 ratio within each age group to receive study medication or placebo but did not describe how the sequence was generated (Griese 2013). The remaining studies did not give any description of how the randomisation sequence was generated.

Allocation concealment

We judged six studies to have a low risk of selection bias (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Conrad 2015; Griese 2013; Howatt 1966; Visca 2015). In the Bishop study, no member of the clinical team was involved in the coding or assignment to treatment or placebo; non-clinical researchers involved in the study were only provided with participant identification numbers, not names (Bishop 2005). In the Calabrese study, the randomisation list was generated by a person not otherwise involved in the study (Calabrese 2015a; Calabrese 2015b). Two studies generated the randomisation numbers centrally and used a telephone system to inform clinicians of participant allocation (Conrad 2015; Griese 2013). In the Howatt study, the drugs were supplied in 10 mL vials labelled with a letter code in a sealed envelope which was not opened until the study was completed (Howatt 1966). In the Visca study, the drug containers were labelled 'A' or 'B' by the pharmaceutical supplier and the blind was removed only after the study had concluded and data analysis begun (Visca 2015).

The risk of bias with respect to allocation concealment is unclear for the remaining 14 studies as it was generally not discussed in the publications (Dauletbaev 2009; Götz 1980; Harries 1971; Homnick 1995b; Keljo 2000; Levin 1961; Mitchell 1982; Portal 1995a; Ratjen 1985; Renner 2001; Sagel 2018; Stafanger 1988; Stafanger 1989; Wood 2003). Levin does state that they concealed the allocation schedules in sealed envelopes, but does not state if these envelopes were opaque, thus the risk of bias is unclear (Levin 1961).

Blinding

We judged there to be a low risk of bias with respect to blinding for 11 studies (Bishop 2005; Conrad 2015; Götz 1980; Howatt 1966; Keljo 2000; Levin 1961; Mitchell 1982; Ratjen 1985; Renner 2001; Sagel 2018; Visca 2015). Six studies matched the taste and smell of treatment and placebo medications to ensure that both participants and the clinical team were blinded (Bishop 2005; Götz 1980; Howatt 1966; Levin 1961; Ratjen 1985; Sagel 2018). Goetz clarified that both substances had a similar taste of orange and were packed in neutral sachets (Götz 1980); Ratjen additionally stated that the colour of the granules was also matched (Ratjen 1985). Three studies stated that the capsules were of identical appearance (Mitchell 1982; Renner 2001; Sagel 2018). In the Conrad study the NAC and the placebo effervescent tablets were packed identically and supplied by BioAdvantex Pharma, Inc. so that all study personnel and participants were blinded to treatment assignment; the codes for each participant were only revealed when data analysis was completed (Conrad 2015). In the Visca study the capsules for both treatments were identical in appearance and in containers labelled 'A' or 'B' by the pharmaceutical supplier (Visca 2015). In the study by Keljo, treatment (naturally occurring RRR-α-tocopherol) and placebo were both provided in vegetable oil (Keljo 2000).

The Dauletbaev study stated that the placebo phase was single-blinded (participants) while the NAC treatment phase was double-blinded (participants and clinicians); it is not clear how this has affected the risk of bias (Dauletbaev 2009). Five studies did not describe the blinding process in enough detail in order to allow a proper assessment of this domain; therefore, the risk of bias with respect to blinding is unclear for these studies (Homnick 1995b; Portal 1995a; Stafanger 1988; Stafanger 1989; Wood 2003).

Three studies were judged to have a high risk of bias from blinding (Calabrese 2015a; Calabrese 2015b; Griese 2013; Harries

1971). The Calabrese study was described by the author as single-blinded because GSH has a distinct taste and smell that is difficult to reproduce as placebo (Calabrese 2015a; Calabrese 2015b). Similarly, although the Griese study was a double-blind study with respect to the packaging of the vials and visual appearance of the medication, those participants treated with GSH could recognize its smell; the authors provide this bias as explanation for the significantly higher dropout rate due to early termination by participant request in the placebo group compared to the treatment group (Griese 2013). In the Harries study, the control group did not receive a placebo but rather no treatment; moreover, the two active interventions used were physically different (tablet versus liquid preparations) (Harries 1971).

Incomplete outcome data

Eight out of 20 studies are judged to have a low risk of attrition bias as the number of withdrawals from the studies as well as the reasons are described in detail for each group (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Conrad 2015; Dauletbaev 2009; Griese 2013; Ratjen 1985; Sagel 2018; Visca 2015).

A further seven studies did not provide a description of withdrawals or dropouts and are judged to have an unclear risk of bias (Götz 1980; Harries 1971; Howatt 1966; Mitchell 1982; Renner 2001; Stafanger 1989; Wood 2003).

The remaining five studies reported incomplete data for the outcomes of interest and have a high risk of bias (Homnick 1995b; Keljo 2000; Levin 1961; Portal 1995a; Stafanger 1988). One study did not described which study arm participants withdrew from; furthermore, the authors of this study did not provide control group data, thereby preventing a comparison between groups in a meta-analysis (Homnick 1995b). Authors were contacted but unable to provide further information because the original data were on a computer they no longer had access to (Homnick 2008 [per comms]). In the paper by Keljo, there are inconsistencies in the number of participants included in each group between the tables of data reporting and the table describing inclusion criteria (Keljo 2000). Furthermore, data from one subgroup of participants are not reported at all due to the very limited number of participants (Keljo 2000). The Levin study, reports that there were 45 participants in the final analysis who had been followed for at least two months and 37 participants who completed the six-month study period (18 in the tocopherol group and 19 in the placebo group); serum tocopherol was reported at two and six months in 18 out of 20 participants initially included in the study and 15 out of 20 participants, respectively (Levin 1961). While the remaining study did not explicitly state the number of participants originally randomised to each group, it is the only study which states the reasons for participant withdrawal (Portal 1995a). In the 1988 Stafanger study, 41 out of 44 participants completed the study, but lung function was only reported for 23 participants (Stafanger 1988).

Selective reporting

Data were reported for all outcomes measured in 12 studies which are judged to have a low risk of bias (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Conrad 2015; Dauletbaev 2009; Götz 1980; Harries 1971; Levin 1961; Mitchell 1982; Portal 1995a; Ratjen 1985; Sagel 2018; Wood 2003).

One study was judged to have an unclear bias due to a lack of information (Howatt 1966).

Seven studies appeared to have a high risk of bias in this domain (Griese 2013; Homnick 1995b; Keljo 2000; Renner 2001; Stafanger 1988; Stafanger 1989; Visca 2015). Griese reported data on cellular and biochemical markers such as GSH and its metabolites in an exploratory manner in a very limited number of participants (Griese 2013). In the Homnick study, authors reported taking measurements at least monthly for 56 weeks, but only present data for baseline and week 50 (Homnick 1995b). In a third study, actual data for BMI were not reported and the difference between groups only described as non-significant; when contacted, the author was unable to provide further data due to relocation of the study statistician (Renner 2001). Stafanger only reports FEV_1 for the 10 participants with baseline peak expiratory flow less than 70% of predicted for sex, age and height (out of 31 participants completing the study) (Stafanger 1989) and lung function results in only 23 out of 41 participants with CF (Stafanger 1988). In the Keljo study, the full paper does not state in the 'Methods' section what the authors planned to report on and the protocol is not available (Keljo 2000). The remaining study does not report spirometry data in the published paper and these data are only available from a poster presentation (Visca 2015). Upon contact for clarification, the corresponding author, Dr. Clark Bishop, has confirmed that the two main reasons were that the pulmonary function was available only for participants over five years of age (representing about half of the treated population) and the lack of a clear physiopathological link between oral supplementation with GSH and improved lung function.

Other potential sources of bias

Two studies included in this review appear to be subject to duplicate publication (Portal 1995a; Renner 2001). In the case of Portal, authors describe the same study in full-length manuscripts, published two years apart (Portal 1995a). The journals in which they are published appear related, but are independent – *Clinical Chemistry* and*Clinica Chimica Acta* (*International Journal of Clinical Chemistry*). Although the two reports appear to describe different outcomes of the same study based on their titles (the 1993 paper reports on biological indices of selenium status and the 1995 paper reports on lipid peroxidation markers), the later paper does not reference the methods already reported in the earlier report. Although the earlier report assesses two outcomes not later described and the latter report describes two not previously described, there is an overlap of two outcomes; neither of which is referred to as having already been reported. As such, the two studies were taken as one here since the outcomes of interest were contained in both studies and the authors of this review did not want to 'double count' participants (Portal 1995a). Another study appeared in the literature in seven different instances - three full-text reports and four abstracts (Renner 2001). At the screening stage of this review, one full-text report was included and the other two were excluded on the basis of unstated diagnostic criteria. Eventually, data from all reports were included for meta-analysis according to Cochrane policy. None of the full-text reports referenced the others and all are reported as 'original' publications.

There is an unclear risk of bias for the Keljo study which has not been published in a peer-reviewed journal; an abstract was presented at the North American Cystic Fibrosis Conference and

additional data presented in this review were supplied directly by the authors (Keljo 2000).

With the exception of two studies which include 153 (Griese 2013) and 105 participants (Calabrese 2015a; Calabrese 2015b), most studies in this review suffer from relatively small sample sizes, ranging from eight (Howatt 1966) to 70 participants (Conrad 2015).

Another source of potential bias occurs in one of the cross-over studies included in this review, where the authors described a proper cross-over regimen, with each arm lasting five months with a two-month washout period between treatment periods (Portal 1995a). However, they failed to measure and report baseline measurements for all outcomes after the washout period and before the start of the second period (Portal 1995a). This prevented the authors of this review from assessing whether a 'carry-over' effect occurred; data from the second period were incomplete and hence could not be included for analysis in this review. In a further cross-over study, a two-week washout period between the three-month treatment periods was registered (Mitchell 1982). In four cross-over studies no washout period between the periods of placebo and treatment administration were reported, but the participants were assessed before and after each treatment period (Howatt 1966; Götz 1980; Stafanger 1988; Stafanger 1989).

In the Griese study which compares the effect of inhaled GSH to placebo, a number of participants were allowed to continue the oral administration of NAC, which is a precursor of GSH and these participants could not be identified from the report. We are therefore unable to assess the possible influence of this treatment on the results (Griese 2013).

Conrad includes 70 participants with CF who belong to two different cohorts; the Stanford cohort of 16 participants who initially entered the study for the safety investigation with a focus on pulmonary arterial hypertension and a second cohort of 54 participants from 10 other CF centres who were enrolled at a later stage after the safety study showed no signs of pulmonary hypertension at eight weeks of NAC treatment (Conrad 2015).

In the study by Visca, there were more participants homozygous for delta F508 (known to have a more severe disease manifestation than heterozygotes) in the placebo group (27.7%) compared to the GSH group (13.6%) (Visca 2015).

We judge the Homnick study to be at a high risk of bias since the authors do not describe baseline demographics and do not state a sample size calculation. Furthermore, investigators did not systematically control dose levels throughout the study (Homnick 1995b).

Effects of interventions

See: **Summary of findings for the main comparison** Summary of findings: oral antioxidants (NAC/GSH) compared to placebo; **Summary of findings 2** Summary of findings: oral vitamin E supplementation versus placebo or no treatment; **Summary of findings 3** Summary of findings: oral β-carotene compared to placebo; **Summary of findings 4** Summary of findings: oral antioxidant combination compared to control; **Summary of findings 5** Summary of findings: oral antioxidant mixed supplement compared with control; **Summary of findings 6** Summary of findings: inhaled antioxidants compared with placebo

In the summary of findings tables, the quality of the evidence has been graded for pre-defined outcomes (see above) and definitions of these gradings provided (Summary of findings for the main comparison; Summary of findings 2; Summary of findings 3; Summary of findings 4; Summary of findings 5; Summary of findings 6).

Oral antioxidant supplementation versus control

Primary outcomes

1. Lung function tests

a. FEV_1

Eight studies (n = 322) reported FEV_1 (% predicted) compared to baseline at a range of time points up to six months (Conrad 2015; Dauletbaev 2009; Ratjen 1985; Renner 2001; Sagel 2018; Stafanger 1989; Visca 2015; Wood 2003). Results are presented in the graphs, but we were not able to generate a total summary statistic as one study contributed data at more than one time point (Analysis 1.1).

After two months of a combined supplement, Wood reported a significant difference in favour of control, MD -4.30% (95% CI -5.64 to -2.96) (Wood 2003).

Four studies comparing GSH or NAC to control reported data at three months (Conrad 2015; Dauletbaev 2009; Ratjen 1985; Stafanger 1989). When data were combined, the intervention group showed a better lung function compared to placebo, though no statistical significance was achieved, MD 2.83% (95% CI -2.16 to 7.83). However, I^2 was 49% (moderate heterogeneity) and it should be underlined that the dosage of NAC in two studies was 600 mg/daily divided in three doses (Ratjen 1985; Stafanger 1989), while in the remaining studies the doses were 2700 mg daily (Conrad 2015) and 2800 mg daily (Dauletbaev 2009) divided in three and four doses, respectively. The quality of evidence for this outcome was deemed to be very low (Summary of findings for the main comparison).

After four months of an antioxidant-enriched vitamin supplement or the vitamin supplement alone, Sagel reported no difference between groups, MD 1.44% (95% CI -2.23 to 5.11) (Sagel 2018).

Three studies report FEV_1 (% predicted) at six months; one evaluating NAC (Conrad 2015), one evaluating oral GSH (Visca 2015) and one evaluating β-carotene (Renner 2001). Both Conrad and Visca individually showed a positive effect of NAC, MD 4.38% (95% CI 0.89 to 7.87) and GSH administered as L-glutathione, MD 17.40% (95% CI 13.97 to 20.83). The different bioavailabilities of NAC and GSH do not support the combination of results from these two studies. The quality of evidence for the effect of NAC was deemed to be moderate and only downgraded because of small number of participants (Summary of findings for the main comparison). At six months, Renner showed no statistical difference between the β-carotene and control groups (Renner 2001); but the quality of this evidence was found to be very low (Summary of findings 3).

Three further studies of oral NAC reported information for FEV_1 which could not be included in the meta-analysis (Götz 1980; Mitchell 1982; Stafanger 1988). Two studies reported a non-significant improvement of FEV_1 during oral administration of NAC (Götz 1980; Mitchell 1982). The third study, however, reported a significant improvement of the FEV_1 after oral administration of

NAC, but this was in a subgroup of participants treated during autumn, when infections are more common (Stafanger 1988).

b. FVC

Five studies (n = 208) reported on FVC (% predicted) compared to baseline at two, three and six months (Conrad 2015; Dauletbaev 2009; Stafanger 1989; Visca 2015; Wood 2003). Results are presented in the graphs, but we were not able to generate a total summary statistic as one study contributed data at more than one time point (Analysis 1.2).

After two months, there was no statistical difference between the combined supplement and control, MD -4.20% (95% CI -11.28 to 2.88) (Wood 2003).

Three studies provided data for NAC versus control at three months and showed a result in favour of NAC, although this was not statistically significant, MD 3.34% (95% CI -4.30 to 10.97) (Conrad 2015; Dauletbaev 2009; Stafanger 1989).

Two studies reported FVC (% predicted) at six months (Conrad 2015; Visca 2015). When GSH was administered as L-glutathione the result significantly favoured GSH, MD 14.80% (95% CI 10.07 to 19.53); however, when administered as NAC the result was non-significant, MD 3.75% (95% CI -0.13 to 7.63).

2. QoL

Data for this outcome were available from two studies (n = 108); one study assessed QoL using the Quality of Wellbeing scale (QoWB) (Wood 2003) and the second used the CF Quality of Life Questioannaire respiratory domain scale CFQ-R (Conrad 2015). We present the results from both studies on the same graph and have therefore analysed the data using the SMD. Results from the Wood study significantly favoured control over antioxidant supplementation at two months, SMD -0.66 points (95% CI -1.26 to -0.07) (Analysis 1.3). In the second study, the CFQ-R was used and reported no significant difference in results between NAC and placebo groups at three months, SMD 0.33 (95% CI -0.17 to 0.83) and at six months, SMD -0.03 (95% CI -0.53 to 0.47) (Conrad 2015). This evidence at six months was of moderate quality but was downgraded due to imprecision from the small number of participants (Summary of findings for the main comparison).

Secondary outcomes

1. Oxidative stress

a. Lipid peroxidation

Four studies reported this outcome (n = 170), one comparing selenium to control (Portal 1995a), one comparing β-carotene to control (Renner 2001), one comparing a combined supplement to control (Wood 2003) and one comparing antioxidant-enriched multivitamins with multivitamins alone (Sagel 2018). These studies reported different measures of lipid peroxidation:

- H_2O_2 (Portal 1995a);
- plasma 8-iso-prostoglandin $F_{2\alpha}$ (Wood 2003);
- malondialdehyde either as TBARS (Portal 1995a; Sagel 2018) or by HPLC (Renner 2001); and
- urine and sputum 8-iso-$PGF_{2\alpha}$ and sputum 8-hydroxydeoxyguanosine (8 -OHdG) (Sagel 2018).

When the data were analysed there was no significant difference at any time point between groups for H_2O_2, MD 15.90 (95% CI -13.16 to 44.96) (Analysis 1.4); plasma F_2-isoprostanes, MD 1.00 (95% CI -23.94 to 25.94) (Analysis 1.5); malondialdehyde, MD -0.10 (95% CI -0.45 to 0.25) (Analysis 1.6); urine and sputum 8-iso-$PGF_{2\alpha}$, MD 0.09 (CI 95% -0.10 to 0.28) and MD 0.02 (95% CI -0.12 to 0.16), respectively (Analysis 1.7; Analysis 1.8); or sputum 8 -OHdG, MD -0.07 (95% CI -0.24 to 0.10) (Analysis 1.9). Due to different measurement units TBARS data from the Sagel study could not be used in the meta-analysis (Sagel 2018).

b. Antioxidant enzyme function

Two studies contributed data for this outcome (n = 73); one of combined supplementation with data reported at two months (Wood 2003) and one of selenium supplementation reported at five months (Portal 1995a). There was a significant improvement in GPX for both combined supplementation, MD 1.60 units per gram of haemoglobin (U/g Hb) (95% CI 0.30 to 2.90) and for selenium supplementation, MD 10.20 U/g Hb (95% CI 2.22 to 18.18) (Analysis 1.10). This was not significant when combined, MD 4.96 U/g Hb (95% CI -3.26 to 13.19), and there was considerable heterogeneity (I^2 = 77%), probably due to the different supplements.

Only the study of combined supplements reported on superoxide dismutase (SOD) at two months and analysis showed that there was no significant difference between groups, MD 0.27 (95% CI -1.24 to 1.78) (Analysis 1.11).

c. Potency

One study of β-carotene supplementation reported on antioxidant potency using trolox equivalent antioxidant capacity (TEAC) as an outcome measure (Renner 2001). At six months, there was no significant different found between supplement and placebo groups, MD 0.04 (95% CI -0.17 to 0.25) (Analysis 1.12).Two studies reported on total plasma antioxidant capacity, though by different methods which did not allow combined analysis; neither study found a significant difference between intervention and control groups at any time point (Renner 2001; Sagel 2018). Renner reported plasma total antioxidant capacity at three and six months after supplementation with β-carotene, MD 0.10 nmol (95% CI -0.18 to 0.38) and MD 0.04 nmol (95% CI -0.30 to 0.38), respectively (Analysis 1.13). Sagel reported at one and four months after supplementation with a multivitamin with an additional antioxidant or just a multivitamin: at one month, MD 0.00 (log (10) CRE) (95% CI -0.02 to 0.02); and at four months, MD -0.01 (log (10) CRE) (95% CI -0.04 to 0.02) (Analysis 1.14).

d. Plasma and sputum antioxidant status

i. Vitamin E

Five studies (n = 224) provided data for this outcome (Harries 1971; Levin 1961; Sagel 2018; Visca 2015; Wood 2003). Two studies supplemented antioxidants in form of vitamin E as D,L-α-tocopheryl acetate (Harries 1971; Levin 1961); Harries supplemented with both fat-soluble and water-miscible forms of vitamin E (Harries 1971). Wood supplemented vitamin E as RRR-α-tocopherol as part of a combined antioxidant supplement (Wood 2003) and Sagel supplemented vitamin E together with other antioxidants in a multivitamin supplement (Sagel 2018). A further study supplemented with oral GSH (Visca 2015). In the blood, vitamin E binds to lipoproteins and therefore reporting the ratio

between the vitamin E levels and total lipids is more accurate than the plasma vitamin E levels, However, only the measurement of plasma vitamin E levels was common to all the included studies.

The supplementation led to significantly increased plasma levels of vitamin E in favour of treatment for all supplements at all time points as follows (Analysis 1.15); however, please note that the control group in the Harries study is the same group of participants in the comparison of fat-soluble vitamin E versus control and water-miscible vitamin E versus control (Harries 1971) (see Table 3). The difference between oral GSH and control groups was less than the supplements containing vitamin E; however, the effect of oral GSH supplementation on the serum levels of vitamin E is indirect (increase regeneration of the oxidized vitamin E) compared to the direct supplementation with vitamin E.

One study included in the review reported changes in the serum vitamin E levels without SDs which make them unsuitable for the meta-analysis (Keljo 2000). The study reported that levels increased from 28.2 to 35 µM/L with vitamin E treatment and from 25.4 to 28.6 µM/L in the placebo group. Baseline serum vitamin E levels were not reported and the paper states "the baseline serum α-tocopherol level did not differ between the placebo and α-tocopherol groups, and there was no difference in the baseline α-tocopherol levels between subgroups (data not shown)".

ii. β-carotene

One study (n = 46) included β-carotene as part of a combined antioxidant supplement (Wood 2003), two studies included it as a single supplement (Homnick 1995b; Renner 2001) and one study investigating an antioxidant-enriched multivitamin supplement containing mixed carotenoids (lutein, zeaxanthin and lycopene) also reported on β-carotene levels (Sagel 2018). However, only two studies (n = 70) presented data suitable for analysis (Renner 2001; Wood 2003). There was a significant improvement in β-carotene levels in favour of both combined supplementation at two months, MD 0.10 µmol/L (95% CI 0.02 to 0.18) and single β-carotene supplementation at six months, MD 0.24 µmol/L (95% CI 0.02 to 0.46) (Analysis 1.16). When combined, the results from all supplements were also significant, MD 0.13 (95% CI 0.02 to 0.25) with only low heterogeneity (I^2 = 27%).

Sagel reported a significantly higher level of β-carotene was reported at four months with a mean change from baseline of 0.04 µg/mL in the group taking the antioxidant-enriched multivitamin supplement (Sagel 2018). Homnick reported that the mean (SD) serum levels of β-carotene increased significantly from baseline 0.09 (0.02) µmol/L to 0.62 (0.19) µmol/L during supplementation with β-carotene; however, no data are available for the β-carotene serum levels in the control group and therefore these results could not be included in the analysis (Homnick 1995b). It is stated in the paper that "no control patient had a significant increase in β-carotene levels throughout the duration of the study" and a mean (SD) baseline of 0.12 (0.05) µmol/L is given, but it is not clear which participants are included.

iii. Selenium

Two studies (n = 73) supplemented selenium (Portal 1995a; Wood 2003). Both the combined supplementation at two months, MD 0.60 µmol/L (95% CI 0.39 to 0.81) (Wood 2003) and the single supplementation at five months, MD 0.39 µmol/L (95% CI 0.27 to 0.51) (Portal 1995a) showed a significant improvement in plasma selenium status in favour of antioxidant supplementation (Analysis 1.17). The combined results from the two studies were also significant, MD 0.48 µmol/L (95% CI 0.27 to 0.68) but with substantial heterogeneity (I^2 = 65%).

iv. Vitamin C

One study (n = 46) supplemented vitamin C as part of a combined antioxidant supplementation (Wood 2003); there was no significant difference in improvement between antioxidant and control, MD 8.00 µmol/L (95% CI -15.05 to 31.05) (Analysis 1.18).

v. GSH in plasma and sputum

One study (n = 61) comparing oral NAC to placebo reported on the change from baseline in GSH in whole blood which we are reporting here (Conrad 2015); there was no difference found between groups at either three months, MD 19.00 µmol/L (95% CI -183.58 to 221.58) or at six months, MD 64.10 (95% CI -170.05 to 298.25) (Analysis 1.19). In a further study (n = 21) NAC was administered in a high dose of 2800 mg for 12 weeks and the authors reported on extracellular glutathione in induced sputum and blood plasma compared to baseline (Dauletbaev 2009). The median (range) value of total glutathione in induced sputum increased from 18.6 µM (2.8 to 32.14) to 31.3 µM (0.2 to 44.3) but this difference did not reach statistical significance. Concentrations of extracellular total glutathione in blood plasma were measured using medians (range) at baseline 1 µM (0.9 to 1.3) and end of treatment 1.4 µM (1 to 1.9). Due to the lack of clarity of the measurements (extracellular total glutathione in blood plasma in the paper and total blood glutathione in the additional data received from the author), as well as due to apparently a different measurement method, these data have not been used in the analysis.

e. Plasma fatty-acid status

One study (n = 46) of a combined antioxidant supplementation examined this outcome (Wood 2003); at two months the data showed no significant difference between groups, MD 166.00 mg/L (95% CI -61.38 to 393.38) (Analysis 1.20).

2. Inflammation

a. Inflammatory markers (i.e. IL-6, IL-8, TNF-α, IL-1β)

Keljo measured plasma levels of IL-6 and TNF-α at three months in three subgroups of participants defined according to lung function and treatment with dornase alfa (DNase) (Keljo 2000). The results from our analyses (Analysis 1.21; Analysis 1.22) are summarised in the additional tables (Table 4). Only the result for TNF-α (pg/mL) in 11 participants with FEV_1 measurements between 70% and 85% taking DNase was statistically significant (in favour of the antioxidant) (Analysis 1.22).

Conrad reported on the change from baseline in plasma IL-8 pg/mL (log 10) and found no difference between groups at three months, MD 0.01 pg/mL (log 10) (95% CI -0.19 to 0.21) or six months, MD -0.09 pg/mL (log 10) (95% CI -0.32 to 0.14) (Analysis 1.23).

Three studies reported on sputum levels of IL-8 (pg/mL) (Conrad 2015; Dauletbaev 2009; Sagel 2018). Two studies compared oral NAC to control at three months (Conrad 2015; Dauletbaev 2009); data showed no significant difference between groups at this time point, MD -0.01 pg/mL (95% CI -0.15 to 0.14) (Analysis 1.24). Sagel

(multivitamin enriched with antioxidant versus a multivitamin alone) also found no difference between groups at four months, MD -0.06 pg/mL (95% CI -0.24 to 0.12), as did Conrad at six months, MD 0.19 (95% CI -0.03 to 0.41) (Analysis 1.24).

Conrad also assessed sputum neutrophil count and found no difference between oral NAC or control groups at either three months, MD 1.90 (95% CI -8.08 to 11.88) or six months, MD 2.60 (95% CI -11.85 to 17.05) (Analysis 1.26). Dauletbaev assessed the number of leukocytes in induced sputum at three months and reported that the number of leukocytes (which were predominantly neutrophils) did not change significantly during NAC treatment (Dauletbaev 2009). The total number (median (range)) of leukocytes was 31.5 (20 to 113.7) x 10^6 at the start of the treatment with NAC 2800 mg/daily, 48.6 (42.6 to 186.2) x 10^6 after three weeks of treatment and 36.8 (19.9 to 110.8) x 10^6 after additional nine weeks of high-dose oral NAC. The results from these two studies were though not suitable for combination due to different units of measurement.

Conrad reported the change from baseline in sputum human neutrophil elastase (log 10) (mg/mg) per weight at three months, MD -0.04 (95% CI -0.24 to 0.16) and six months, MD 0.11 (95% CI -0.11 to 0.33); neither result was statistically significant (Analysis 1.25).

The study comparing a multivitamin enriched with an antioxidant and a control multivitamin preparation reported sputum myeloperoxidase (MPO) levels at four months, but found no difference between groups, MD -0.13 (log 10) (ng/mL) (95% CI -0.48 to 0.22) (Analysis 1.27).

b. Hyperinflation of chest

No studies examined this outcome.

3. Nutritional status

a. BMI

One study comparing NAC to placebo reported the change from baseline in BMI at three and six months (Conrad 2015). There were no differences between groups at either three months, MD 0.30 (95% CI -0.02 to 0.62), or at six months, MD 0.20 (95% CI -0.23 to 0.63) (Analysis 1.28). The evidence for this outcome at six months is of moderate quality and was only downgraded due to imprecision from the small number of participants (Summary of findings for the main comparison).

One study measured the effects of supplementing β-carotene on BMI, but only reported baseline values and stated that there was a non-significant effect of supplementation on this outcome (Renner 2001). We were unable to obtain full data for this outcome from the study investigators.

b. BMI percentile

One study reported on the change in BMI percentile after three and six months of oral supplementation with GSH (Visca 2015). BMI percentile increased significantly more with GSH supplementation than control after both three months, MD 9.20% (95% CI 6.22 to 12.18) and after six months, MD 17.20% (95% CI 14.35 to 20.05) (Analysis 1.29). The quality of this evidence was found to be moderate and was downgraded due to imprecision from the small number of participants (Summary of findings for the main comparison).

c. Weight

Three studies reported on the change in weight from baseline measured in kg at three and six months (Conrad 2015; Levin 1961; Mitchell 1982). Combined data from the Conrad and Mitchell studies (both comparing NAC to control) at three months showed no significant difference between groups, MD 0.24 kg (95% CI -0.73 to 1.22); but there was substantial heterogeneity (I^2 = 66%). This is probably due to differences in the standard of CF care in 1982 and 2015 (Conrad 2015; Mitchell 1982). The Conrad study also showed no significant difference between NAC treatment and placebo at six months, MD 0.60 kg (95% CI -0.51 to 1.71). Additionally, Levin showed no difference between vitamin E supplementation and placebo at six months, MD -0.30 kg (95% CI -7.19 to 6.59) (Analysis 1.30).

d. Weight percentile

One study reported on the change in weight percentile from baseline after three and six months of GSH supplementation (Visca 2015). Weight percentile increased significantly more with GSH than control at three months, MD 8.10% (95% CI 5.64 to 10.56) and also at six months, MD 17.00% (95% CI 14.64 to 19.36) (Analysis 1.31).

Sagel reported that at four months there was no difference in weight z scores between the intervention and control group; the 16-week difference in unadjusted weight z score was 0.07 (95% CI -0.10 to 0.25; P = 0.41) (Sagel 2018).

4. Antibiotic days

The number of antibiotic days per participant in both treatment groups was reported in two studies (n = 70) (Renner 2001; Wood 2003). No significant difference between groups was found either after two months of a combined supplement, MD 4.00 (95% CI -14.06 to 22.06), or after six months of β-carotene supplementation, MD -8.00 (95% CI -18.78 to 2.78) (Analysis 1.32). The combined result was also not significant, MD -4.28 (95% CI -15.16 to 6.60).

Two studies reported the number of participants with pulmonary exacerbations requiring antibiotics (Conrad 2015; Sagel 2018). There was no difference in the comparison of multivitamins with antioxidant to multivitamin alone at four months, RR 0.78 (95% CI 0.53 to 1.14), or in the comparison of NAC to control at six months, RR 0.83 (95% CI 0.50 to 1.390, or when combined, RR 0.80 (95% CI 0.59 to 1.09) (Analysis 1.33). Conrad also reported no difference in the number of participants hospitalised, RR 0.94 (95% CI 0.49 to 1.81) (Analysis 1.34). Sagel analysed the risk of first pulmonary exacerbation using a co-variate-adjusted hazard ratio (adjusted to account for a higher number of participants over the age of 30 in the control group); we present the result directly from the paper. There was a significantly lower risk of first pulmonary exacerbation in the antioxidant group than the control group at four months, HR 0.5 (95% CI 0.25 to 0.98) (P = 0.04) (Sagel 2018). This evidence was deemed to be low quality due to risk of bias within the study and low event rates.

5. Adverse events

While it was possible to identify specific adverse events, the rates of specific events were not calculable due to inadequate reporting. A total of 11 studies reported on adverse events or deaths during the study (Conrad 2015; Dauletbaev 2009; Götz 1980; Keljo 2000;

Levin 1961; Mitchell 1982; Portal 1995a; Renner 2001; Sagel 2018; Stafanger 1989; Visca 2015).

Adverse events

Two parallel studies reported that no side-effects were noticed in either the placebo or the active treatment (both of which were NAC) (Götz 1980; Mitchell 1982) and one cross-over study of β-carotene reported that no adverse events occurred (Renner 2001); but we deemed the quality of this evidence to be very low.

We were able to analyse adverse event data from three studies; one three-month study assessing a form of vitamin E (Keljo 2000; low-quality evidence (Summary of findings 2)), one four-month study assessing a multivitamin enriched with an antioxidant (Sagel 2018; low-quality evidence; Summary of findings 5) and one six-month study assessing NAC (Conrad 2015; moderate-quality evidence; Summary of findings for the main comparison). None of the results were statistically significant (Analysis 1.35). All three studies reported on sinusitis, OR 1.58 (95% CI 0.38 to 6.55), distal intestinal obstruction syndrome, OR 0.47 (95% CI 0.09 to 2.34) and diarrhoea, OR 1.76 (95% CI 0.58 to 5.32). Two studies reported on pulmonary exacerbations, OR 0.57 (95% CI 0.23 to 1.41) (Keljo 2000; Sagel 2018); and two studies reported on elevated liver enzymes, OR 3.04 (95% CI 0.31 to 30.19) (Conrad 2015; Keljo 2000). Conrad reported a lack of signs of pulmonary hypertension, which was the focus of the safety study in the first eight weeks of the study and which included a subset of 16 participants from the Stanford CF Center. Additional data obtained after contacting the author presented various adverse effects, especially gastrointestinal, that have been used in the analysis (Analysis 1.35)

Dauletbaev reported comparable numbers of mild to moderate adverse effects in both groups (NAC 700 mg/daily or 2800 mg NAC/daily) most of which were exacerbations of CF lung disease. There were three adverse effects rated as serious, but these were not considered to be related to the study medication (two polypectomia and one haemoptysis during common cold) (Dauletbaev 2009). One adverse effect (gastrointestinal bleeding) was considered to have a "possible" relationship to the medication (Dauletbaev 2009).

In the 1989 study, Stafanger reported the number of people with adverse events which led to them being excluded from the study (Stafanger 1989). Investigators reported that one individual in the NAC group developed Quincke's oedema and one developed exanthema; in both cases symptoms disappeared when the treatment was stopped. Two participants complained of abdominal pain, one from the NAC group and one from the placebo group. One participant complained of more frequent coughing that was less productive while taking NAC (Stafanger 1989).

The Visca study presented participants' self-reported qualitative gastrointestinal symptoms (abdominal pain, belching, flatulence, lack of appetite, bloating, nausea. vomiting, heartburn, diarrhoea and bowel movements (more than twice-daily or less than twice-weekly)) during the course of the study and divided the participants in groups with improved symptoms, no change or worsened symptoms (Visca 2015). The investigators reported a trend towards improvement of the symptoms in participants treated with GSH.

Deaths

Keljo reported there were no deaths during the study (Keljo 2000). One of the cross-over studies stated that one death occurred in the group which received selenium first followed by placebo; however, investigators did not state a time point or period during which the death occurred, other than to say that only baseline data were used in the analysis (Portal 1995a). Another study of vitamin E reported three deaths, all of which were in the control group (Levin 1961).

Inhaled antioxidant supplementation versus control

One study reported separate data for a pediatric cohort and an adult cohort; in order to present these data separately we have generated two study IDs for one single study, one for the adult data (Calabrese 2015a) and one for the pediatric data (Calabrese 2015b).

Primary outcomes

1. Lung function tests

a. FEV_1

Four out of five studies of inhaled GSH and NAC report data on FEV_1 (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013).

i. Change from baseline FEV_1 (L)

Two studies provided data for this outcome (Calabrese 2015a; Calabrese 2015b; Griese 2013). Calabrese reported data at one, three, six and nine months separately for adults (18 years and older) and children (age between 6 and 18 years) and these have been entered separately in our analysis (Calabrese 2015a; Calabrese 2015b). Based on the mean age of the participants included in the study of Calabrese, the data of the adult group (mean (SD) age 28.9 (9.4) years) were chosen to be combined to the data from the study of Griese (mean (SD) age 23.1 (9.8) years) in a secondary analysis to assess the effect of age on the results. In the original paper, Griese reported graphically the change from baseline in FEV_1 (L) at one, three and six months (Griese 2013).

At one month, the combined data from all participants reporting on the change from baseline FEV_1 (L) showed no significant difference between groups, MD 0.05 L (95% CI -0.01 to 0.11). This remained non-significant when the pediatric data were removed and just the adult data combined, MD 0.05 L (95% CI -0.02 to 0.11). At three months, results for all participants significantly favoured the inhaled GSH group, MD 0.09 L (95% CI 0.03 to 0.15) and remained so when the pediatric data were removed, MD 0.09 L (95% CI 0.02 to 0.16). At six months, data for all participants just significantly favoured the antioxidant group, MD 0.07 L (95% CI 0.00 to 0.14), but became non-significant when the pediatric data were removed, MD 0.06 L (95% CI -0.01 to 0.14). At nine months, only Calabrese reported data for this outcome; results were not significant for either the adult population or the pediatric population or combined data, MD 0.03 L (95% CI -0.14, 0.20). Similarly at 12 months, only Calabrese reported data for this outcome; and again results were not significant for either the adult population or the pediatric population or combined data, MD -0.00 (95% CI -0.13 to 0.12) (Analysis 2.1).

ii. Change from baseline FEV_1 (% predicted)

Two studies provided data for the change from baseline (% predicted) in the published papers (Bishop 2005; Calabrese 2015a;

Calabrese 2015b). The first author of the Griese paper provided values for FEV_1 % predicted on request (Griese 2013).

At one month, data from all participants in the Calabrese and Griese studies were not statistically significant, MD 1.91% (95% CI -0.07 to 3.88); this was also true when the data from just the adult participants from the Calabrese study were combined with the Griese study, MD 1.66% (95% CI -0.41 to 3.72) (Analysis 2.2). There were also no differences between groups in the Bishop study at two months, MD 0.90% (95% CI -6.45 to 8.25). At three months data from all participants in the Calabrese and Griese studies significantly favoured the antioxidant group, MD 3.50% (95% CI 1.38 to 5.62), which remained when the pediatric data were removed, MD 3.68 (95% CI 1.17 to 6.19). We rated the quality of this evidence to be moderate; downgraded once due to risk of bias in the included studies (Summary of findings 6). Combined six-month data for all ages showed no difference between groups, MD 2.30% (95% CI -0.12 to 4.71), nor did the combined data from Calabrese adult population and the Griese study, MD 2.17% (95% CI -1.07 to 5.41) (Analysis 2.2). Again, we deemed the quality of this evidence to be moderate (Summary of findings 6). Only Calabrese reported data at nine and 12 months; data for the combined adult and pediatric populations showed no difference between treatment and control, at nine months, MD 2.52% (95% CI -4.61 to 9.65) and at 12 months, MD 2.96% (95% CI -2.54 to 8.46). However, results were significantly in favour of GSH for the adult population at both nine months, MD 5.47% (95% CI 0.97 to 9.97) and 12 months, MD 5.45% (95% CI 0.46 to 10.44) (Analysis 2.2).

b. FVC

Three studies provided data for FVC (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013). Investigators on the Calabrese study provided values for FVC in L and % predicted upon request (Calabrese 2015a; Calabrese 2015b); likewise values for FVC % predicted were obtained from the first author of the Griese study after contact (Griese 2013).

i. Change from baseline FVC (L)

Data from the Calabrese (whole population) and Griese studies showed no differences between groups in FVC (L) at one month, MD 0.05 L (95% CI -0.01 to 0.12), results were also non-significant when we analysed just the adult population from Calabrese with the Griese data (Calabrese 2015a; Griese 2013). At three months, results for the whole population were just significant in favour of antioxidant supplementation, MD 0.08 L (95% CI 0.01 to 0.16), but non-significant when the pediatric data were removed, MD 0.07 L (95% CI -0.01 to 0.15). At six months, there was no difference between groups for the whole population, MD 0.05 L (95% CI -0.03 to 0.13) or for the adult population only, MD 0.02 L (95% CI -0.07 to 0.12) (Analysis 2.3). Only Calabrese reported data at nine and 12 months and again there were no differences for the whole cohort at either time point; nine months, MD 0.01 L (95% CI -0.17 to 0.19) and 12 months, MD -0.01 L (95% CI -0.10 to 0.09); results were also non-significant for the individual age groups (Analysis 2.3).

ii. Change from baseline FVC (% predicted)

All three studies provided data for the change from baseline in FVC % predicted (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013). At one month, results from Calabrese (whole population) and Griese showed no difference between groups, MD 2.12% (95% CI -0.23 to 4.47); this was also true when the paediatric results from the Calabrese study were removed from the analysis, MD 3.02% (95% CI -1.89 to 7.92) (Analysis 2.4). Only Bishop reported data at two months and this result was also not statistically significant, MD 0.60% (95% CI -6.53 to 7.73). However, at three months data from Calabrese and Griese showed a significant difference in favour of the antioxidant for all participants, MD 3.60% (95% CI 1.33 to 5.88). When we considered the different age groups from the Calabrese study, the paediatric data, MD 5.06% (95% CI -0.28 to 10.40) were non-significant, but the adult data (both studies) did show a difference, MD 3.28% (95% CI 0.77 to 5.79) (Analysis 2.4). At six months, results were again non-significant both with the pediatric data, MD 3.33% (95% CI -0.62 to 7.27) and when just considering the adult data, MD 2.71% (95% CI -2.64 to 8.07) and also for all participants combined, MD 3.33% (95% CI -0.62 to 7.27). Only Calabrese reported data at nine months when the overall result and the pediatric data were non-significant, MD 5.48% (95% CI -1.76 to 12.73) and MD 1.46% (95% CI -6.45 to 9.38) respectively; however, the adult-only data were statistically significant, MD 8.88% (95% CI 2.18 to 15.59). At 12 months again only Calabrese provided data; these were not statistically significant when combined, MD 4.27% (95% CI -0.00 to 8.54), results for both age groups were also not statistically significant (Analysis 2.4).

2. QoL

Three studies reported on QoL (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013). Two studies used different versions of the validated CFQoL - Calabrese used the Italian version (Calabrese 2015a; Calabrese 2015b) and Griese used the German version (Griese 2013).

Griese reported the change from baseline in total score and found no significant differences between groups at one month, MD 2.20 (95% CI -0.23 to 4.63), three months, MD 1.20 (95% CI -1.46 to 3.86) and six months, MD 0.80 (95% CI -1.63 to 3.23) (Analysis 2.5). Griese also reported the change from baseline in respiratory score and likewise found no significant differences between groups at one month, MD 2.70 (95% CI -2.15 to 7.55), three months, MD -0.50 (95% CI -4.80 to 3.80) and six months, MD -3.30 (95% CI -8.05 to 1.45) (Analysis 2.5). We rated the quality of this evidence to be moderate and being downgraded due to risk of bias from lack of blinding (Summary of findings 6).

Calabrese reported CF QoL as mean (SD) in GSH and placebo groups at baseline and after 12 months for a range of age groups and found no statistical significant difference (Table 5). As the German and Italian questionnaires assess QoL in different ways and the number of participants in each age group were not provided, the data from Calabrese study could not be used in the meta-analysis.

Bishop used a self-reported scale (not validated), which we do not present in the analysis (Bishop 2005).

Secondary outcomes

1. Oxidative stress

a. hydrogen peroxide (H_2O_2) exhalation

Only one study reported this parameter at 12 months (Calabrese 2015a; Calabrese 2015b). No significant difference between the groups was observed at 12 months, MD -0.16 (95% CI -0.40 to 0.09) (Analysis 2.7).

b. lipid peroxidation (8-isoprostanes) in sputum

Griese measured 8-isoprostane in the sputum of a small number of participants and reported data at three and six months (Griese 2013). Neither result was significant, but the level of lipid peroxidation was lower in the group treated with antioxidants at both three months, MD -51.30 (95% CI -128.22 to 25.62) and six months, MD -5.60 (95% CI -95.70 to 84.50) (Analysis 2.8).

c. antioxidant enzyme function (post hoc change)

None of the studies reported this parameter (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013; Howatt 1966).

d. potency (post hoc change)

None of the studies reported this parameter (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013; Howatt 1966).

e. sputum and plasma antioxidant status

One study measured levels of free and total GSH and its metabolites in sputum (Griese 2013). At both one month and three months, there was no statistically significant difference in free GSH between groups, MD 131.30 pM (95% CI -36.81 to 299.41) and MD 81.40 pM (95% CI -8.01, 170.81), respectively; however, data did show a significant difference in free GSH at six months in favour of the GSH group, MD 59.10 pM (95% CI 3.68 to 114.52) (Analysis 2.9). Conversely, results for total GSH were significant in favour of the GSH group at one and three months, MD 405.30 pM (95% CI 105.27 to 705.33) and MD 329.20 pM (95% CI 167.04 to 491.36) respectively, while the results at six months were not statistically significant, MD 273.50 pM (95% CI -19.52 to 566.52) (Analysis 2.10).

Griese also measured intracellular levels of GSH in neutrophils in the sputum in a small subgroup of participants (eight out of 73 in the GSH group and eight out of 80 in the placebo group) at one, three and six months (Griese 2013). No difference between groups was seen at one month, MD 0.80 mean fluorescence intensity (MFI) (95% CI -0.06 to 1.66), but statistically significant differences between the two groups were found for GSH levels at three and six months, MD 3.70 MFI (95% CI 0.27 to 7.13) and MD 4.40 MFI (95% CI 1.52 to 7.28) respectively (Analysis 2.11).

Griese also measured levels of free and total GSH and its metabolites in plasma, but only at six months (Griese 2013). No statistically significant differences between the two groups were found for either of these levels; free GSH, MD 2.20 pM (95% CI -1.44 to 5.84) and total GSH, MD 0.80 pM (95% CI -2.07 to 3.67) (Analysis 2.12; Analysis 2.13).

The intracellular levels of GSH in neutrophils in the blood were reported by Griese at the six-month time point for a subset of participants (four out of 73 participants in the GSH group and nine out of 80 participants in the placebo group) (Griese 2013). Results were not statistically significant, -2.90 MFI (95% CI -12.39 to 6.59) (Analysis 2.14).

f. plasma fatty acid status

None of the studies reported this outcome (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013).

g. Carbonylated proteins

One study measured carbonylated proteins in the sputum of a subgroup of participants as a marker of oxidative stress (Griese 2013). No statistical significant difference was found for the change from baseline in levels of protein carbonyls in the sputum at one, MD 4.20 U (95% CI -7.92 to 16.32), three, MD -0.10 U (95% CI -13.20 to 13.00), or six months, MD 10.70 U (95% CI -2.63 to 24.03) (Analysis 2.15).

2. Inflammation

a. inflammatory markers (i.e. IL-6, IL-8, IL-10,TNF-α, IL-1β)

One study analysed levels of these cytokines and chemokines in the sputum of a subgroup of participants (24 out of 73 participants in the GSH group and 29 out of 80 participants in the placebo group) at six months (Griese 2013). No statistically significant differences between the two groups in change in levels from baseline were found: IL-8, MD -478.30 pg/mL (95% CI -1536.75 to 580.15) (Analysis 2.16); IL-10, MD -0.20 pg/mL (95% CI -10.12 to 9.72) (Analysis 2.17); and TNF-α, MD 19.80 pg/mL (95% CI -50.33 to 89.93) (Analysis 2.18).

b. hyperinflation of chest

None of the studies reported this outcome (Bishop 2005; Calabrese 2015a; Calabrese 2015b Griese 2013).

3. Nutritional status

a. BMI

Two studies reported analysable data for the change in BMI from baseline to the end of the study (Bishop 2005; Calabrese 2015a; Calabrese 2015b). At two months, no statistically significant difference was found between groups, MD 0.10 (95% CI -0.74 to 0.94) (Bishop 2005) (Analysis 2.19). At 12 months investigators found no difference in BMI between the placebo group compared to the GSH group for the total population, MD 0.04 (95% CI -8.20 to 8.27); this was also true for the separate adult and pediatric populations (Analysis 2.19).

b. Weight

A third study measured the change in weight from baseline to one, three and six months (Griese 2013). There was a statistically significant gain in weight in the GSH group after three months, MD 1.00 kg (95% CI 0.39 to 1.61), but results at one and six months were not significant, MD 0.10 kg (95% CI -0.23 to 0.43) and MD 0.30 kg (95% CI -0.37 to 0.97), respectively (Analysis 2.20).

4. Pulmonary exacerbations requiring intravenous antibiotic therapy or hospitalisation

Two studies reported data on the number of pulmonary exacerbations during the study and also the mean number of days until the first pulmonary exacerbation (Calabrese 2015a; Calabrese 2015b; Griese 2013). There was no difference between groups in the number of exacerbations recorded in the Griese study at six months, MD -0.09 (95% CI -0.30 to 0.12) or in the Calabrese study (total cohort) at 12 months, MD -0.18 (95% CI -0.60 to 0.23) (Analysis 2.21).

The six-month study reported no significant difference between groups in the time to the next exacerbation or the number of exacerbations. The data were skewed and the study authors used an appropriate analysis in their paper; we are unable to reproduce their results without individual patient data (Griese 2013). In the 12-month study (Calabrese 2015a; Calabrese 2015b), there was no statistical difference observed between groups, MD -6.74 days (95% CI -48.76 to 35.27) (Analysis 2.22).

5. Adverse events

The number of participants with specific adverse events were reported by all three studies (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013), but only 'rhinitis/sinusitis or upper respiratory tract infection', 'headache' and 'haemoptysis' were reported by all the three studies (Analysis 2.23). Two of the studies reported on each of the events cough, pharyngitis, stomach pain, chest pain, and nose bleed (Bishop 2005; Griese 2013), but the remainder of the adverse events reported by one study only. None of the adverse reactions showed a statistically significant difference between groups inhaling GSH compared to placebo.

Regarding severity of adverse events, Bishop reported no serious adverse events (Bishop 2005). Calabrese reported that none of the reported adverse events led to discontinuation of the drug and that no death occurred (Calabrese 2015a; Calabrese 2015b). Griese reported that the number of serious adverse events were similar between the group treated with GSH inhalations and the placebo group (11% and 10%, respectively) (Griese 2013).

We assessed the quality of this evidence to be low because of risk of bias within the underlying studies in the blinding domain and low event rates (Summary of findings 6).

Sensitivity Analysis

Since there were so few studies contributing data to the primary outcomes (which we could combine), a sensitivity analysis with regards to risk of bias was not conducted. However, this may be a useful analysis in the future, especially with respect to the high risk of incomplete data and selective reporting which plagues the current review. Sensitivity analyses excluding studies with industry funding was planned but not conducted.

Due to inadequacies of reporting numbers of enrolled participants, completed participants and analysed participants in most studies, an intention-to-treat analysis was not possible.

Publication bias

A funnel plot was not generated, since we were not able to include and combine a sufficient number of studies in this review (Light 1994). Also, only limited data were available for analysis from those included studies.

DISCUSSION

Summary of main results

In the revised version of this review, we chose to extend the list of oral antioxidant micronutrients (vitamin E, vitamin C, β-carotene and selenium) with GSH and NAC (as a precursor of GSH) administered orally. Both NAC or GSH can also be inhaled by people with CF and the effects of the two routes of administration are presented separately. Although NAC and GSH may work as both mucolytics as well as antioxidants, due to the fast hepatic metabolization of oral NAC, the main effect is presumably due to its antioxidant properties.

Oral supplementation

There appears to be conflicting evidence regarding the clinical effectiveness of oral supplementation with antioxidants in CF; however only a small number of studies contributed with data towards meta-analysis. Of the eight studies reporting lung function measured by FEV_1 (Conrad 2015; Dauletbaev 2009; Ratjen 1985; Renner 2001; Sagel 2018; Stafanger 1989; Visca 2015; Wood 2003), four (136 people with CF) provided data on the effect of NAC supplementation on the change from baseline in FEV_1 % predicted at three months which could be combined; results showed no significant improvement (Conrad 2015; Dauletbaev 2009; Ratjen 1985; Stafanger 1989). However, it is important to mention that in these four studies the participants received NAC in differed dosages. In addition, the studies were conducted over 30 years from 1985 (Ratjen 1985) through to 2015 (Conrad 2015). In this period of time, both the standard of CF care and the life expectancy of people with CF have changed dramatically, making the comparison difficult to interpret. At six months, one study showed a significant improvement in FEV_1 % predicted after supplementation with NAC (Conrad 2015) and a further study after supplementation with GSH (Visca 2015). Data from three other studies investigating oral antioxidant supplementation with combined antioxidants, β-carotene or antioxidant-enriched multivitamins did not show an improvement in lung function as reported at two months (Wood 2003), four months (Sagel 2018) or six months (Renner 2001). Due to the short half-life of GSH (Reed 2008), repeated daily dosing for relatively extended time periods (six months) is essential for the effect. One two-month study with 46 participants assessed QoL, and showed that an improvement in QoL actually favoured the control group (Wood 2003). However, no significant differences in CF Quality of Life Questionnaire - Respiratory Domain were reported between NAC and placebo in the Conrad study at either three or six months (Conrad 2015). There was a significant difference between antioxidants and control in both improvement of glutathione peroxidase (GPX) and plasma antioxidant status for all antioxidants except vitamin C. There was an improvement in the blood levels of vitamin E in all studies that used supplementation with this vitamin, as well as in the study with oral GSH supplementation (Visca 2015), although vitamin E was administered in different forms and for different periods of time (Harries 1971; Levin 1961; Sagel 2018; Wood 2003). One study reported significant improvement in the nutritional status (weight percentile and BMI percentile) of children after oral supplementation with GSH (Visca 2015). No study showed any difference between treatment and control in terms of any measure of antibiotic use or pulmonary exacerbations. Adverse events were not adequately reported; there was only one death in a study of 27 participants reported, but this was not clearly attributable to the supplement (selenium) or placebo (Portal 1995a).

Inhaled supplementation

Three studies contributed to the analysis of supplementation with inhaled GSH (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013). The sizes of the Calabrese (n = 105) and the Griese (n = 153) studies are much larger than the Bishop study, which only randomised 19 participants. All studies reported the primary outcome of this review (lung function) at different time points and with a variety of units of measurement. An effect on the change from baseline in FEV_1 % predicted in favour of the antioxidant supplementation was observed at three months by combining results from two studies, which were similar in relation to the included CF population and regimen of administration (Calabrese 2015a; Calabrese 2015b; Griese 2013). However, It has been shown that the final outcome of GSH inhalation therapy might be influenced by the sputum levels of gamma-glutamyltransferase, an enzyme secreted by activated phagocytes that can rapidly degrade

endogenous GSH as well as GSH exogenously administered by inhalations (Corti 2017). All studies in this comparison reported on QoL (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013). Although both Griese and Calabrese reported using validated methods, the methods and the units of measurements were different and therefore, unfortunately not comparable; when analysed individually neither study showed any difference in QoL between treatment and control. The levels of GSH, both the oxidized and reduced form, in the sputum, as well as the intracellular levels in sputum neutrophils were higher in those participants inhaling GSH compared to controls. No differences between the two groups were observed in levels of GSH in the blood (Griese 2013). Three studies reported on different measures of nutritional status (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013). In general there were no differences between treatment or control, but Griese did report a significant increase in weight in the antioxidant group at three months, which was not maintained at six months (Griese 2013). There were no differences in the number of pulmonary exacerbations in two studies, but one of these, a six-month study, did report a significantly longer time frame until the first exacerbation occurred in the antioxidant group (Griese 2013). A wide range of adverse events were reported by three studies (Bishop 2005; Calabrese 2015a; Calabrese 2015b; Griese 2013), the most common being cough, pharyngitis, headache and hemoptysis; there was no difference between treatment or control groups in any adverse event.

Overall completeness and applicability of evidence

Based on the natural history of lung function changes in CF, power calculations demonstrate that approximately 400 participants would need to be enrolled and studied over a six-month period of time if lung function decline, as the primary outcome, was to be halved over the course of the study (Konstan 2010). However, such studies needed to obtain an appropriate power are very difficult to conduct. In addition, a range of different doses of antioxidants have been used in the included studies that have been conducted over a considerable time-span (from 1980s to 2017) characterized by highly different standards of CF care. All these confounders challenge the interpretation of the results.

For oral supplements, eight studies examined the primary outcome of lung function, but relatively few data contributed to the meta-analysis: at three months (four studies). The absence of reporting of methods used to determine sample size in all of the included studies yields questions regarding minimum important difference of outcomes, possibly because these data do not exist for many of the biological markers used as primary outcomes.

There was one cross-over RCT, from which complete data were only reported from the first period, thereby halving the intended sample size and yielding an underpowered study, which makes a significant difference undetectable (Portal 1995a). A completely reported sufficiently-powered study is necessary before concluding that antioxidant supplementation had no effect on lung function. Specifically, investigators did not present baseline measurements for the second treatment period following the wash-out period making assessment of carryover effect unfeasible. The authors of this review acknowledge that since only half of the intended population was included in meta-analysis, issues of reduced power may prevent the study results from revealing true differences between intervention and control. This also contributed to the decision not to pool the treatment effect.

There was evidence that antioxidant supplementation improved plasma status for the respective micronutrient being supplemented. However, the correlation of plasma antioxidant status to clinically important outcome measures in CF has not been adequately explored.

For inhaled supplements, three studies contributed to the analysis and an effect on lung function in favour of the supplements was observed after three months of supplementation (based on two large studies, which used similar inhalation doses and regimen).

A common draw-back of these studies is that the participants are intensively treated with inhaled antibiotics and other treatments that lead to a significant improvement of their lung function making further improvements by addition of antioxidants difficult to assess without very large number of participants.

Quality of the evidence

The quality of the evidence for the clinically important outcomes is presented in the summary of findings tables (Summary of findings for the main comparison; Summary of findings 2; Summary of findings 3; Summary of findings 4; Summary of findings 5; Summary of findings 6).

Oral supplementation

For the comparison of oral antioxidants versus comparator, the quality of evidence is affected by the fact that several different agents were included and could not be combined and so the resulting quality of evidence is lower due to small numbers of studies and few participants contributing to the outcome.

The overall quality of the evidence for the comparison of NAC or GSH was very low to moderate across all of the outcomes. Whilst four studies contributed data to the primary outcome of change in FEV_1 at three months and no significant improvement was seen, the quality of the evidence was graded as being very low because of risk of bias in the underlying studies, particularly around the domains of allocation concealment and blinding, inconsistency of results and imprecision from low event rates. Although only one study contributed data to the change in FEV_1 at six months, there was moderate quality of evidence in favour of the antioxidant (either NAC or GSH supplement) which was downgraded only due to a small sample size. For the same reasons, the quality of evidence for the remaining outcomes was also moderate (Summary of findings for the main comparison). The remaining comparisons of oral supplementation included only one study in each and so the quality of the evidence reflected that of the underlying studies. The quality of the evidence for vitamin E compared to placebo was graded as low due to risk of bias in the study across several domains and only reporting on one of our selected outcomes (adverse events) (Summary of findings 2). Similarly, the comparison of β-carotene only reported on two of our primary and secondary outcomes, FEV_1 % predicted at six months and adverse events, however the quality of this evidence was deemed to be very low due to unclear risk of bias across most domains in the underlying study, imprecision from a small sample size and publication bias (Summary of findings 3). The comparisons of two different mixed combinations of antioxidants versus placebo did not report on any of our clinically important outcomes at the specified time points (Summary of findings 4; Summary of findings 5).

Inhaled supplementation

The quality of the evidence across most of the outcomes for inhaled antioxidants was low or moderate and was downgraded due to the risk of bias within the included studies, particularly around blinding caused by the intervention having a distinctive taste and smell. We found the quality of evidence for adverse events to be low due to low event rates for many of the reported adverse events (Summary of findings 6).

Potential biases in the review process

No articles on the Cochrane Cystic Fibrosis and Genetic Disorders Group's CF Trials Register have been recorded as containing the terms 'vitamin C' or 'glutathione', hence these terms were not searchable keywords in that register. Previously, additional searches of other databases were conducted using these terms (*see* Appendices).

Two studies reported data for the number of antibiotic days (Renner 2001; Wood 2003). Of those, one reported a range rather than a SD (Wood 2003). As such, the SD was imputed using the range yielding an inaccurate estimate, since ranges are distorted by outliers in the data. If the data from this study were to be excluded, the MD between groups would be -23.00 days (95% CI -34.71 to -11.29) (or 23 less days) in favour of antioxidants based on the remaining study (Renner 2001) and may better represent antioxidant effect on this outcome.

Agreements and disagreements with other studies or reviews

The data presented within this systematic review have not been previously synthesized. During the screening phase of this review, numerous case-control and cohort studies on this topic were identified (*see* Characteristics of excluded studies) and such studies have been the basis for clinical trials in this area. Previous studies suggest that antioxidant micronutrients are likely to play a role in the oxidative stress that occurs in CF lung disease and have shown beneficial results (Winklhofer-Roob 1994; Winklhofer-Roob 1997a; Winklhofer-Roob 2003; Wood 2002). However, the aim of this review was to obtain the most rigorous studies on which to base conclusion that have been asserted by multiple cohort and case-control studies to date. In accordance with a previous review on the topic, oral supplementation with antioxidants does not show a beneficial effect on clinical outcomes, but does show an improvement in the laboratory measurements of the supplements (Galli 2012). According to results summarized in this review the administration of inhaled GSH seems to stop the deterioration in lung function in people with CF. This is in contrast to conclusions of a previous Cochrane Review analysing the use of nebulized and oral thiol derivatives, including NAC and glutathione, in people with CF (Tam 2013).

AUTHORS' CONCLUSIONS

Implications for practice

For the oral supplementation, this review and meta-analyses are based on seven studies of different micronutrients (Harries 1971; Keljo 2000; Levin 1961; Portal 1995a; Renner 2001; Sagel 2018; Wood 2003) and seven studies with N-acetylcysteine (NAC) (as a precursor of glutathione (GSH)) (Conrad 2015; Dauletbaev 2009; Götz 1980; Howatt 1966; Ratjen 1985; Stafanger 1988; Stafanger 1989) and one study with oral GSH (Visca 2015); one eligible study contributed with no data (Homnick 1995b). With regards to micronutrients, there does not appear to be a positive treatment effect of antioxidant micronutrients on clinical end-points such as lung function, quality of life (QoL), adverse events. In one study, the time to first pulmonary exacerbation and rate of pulmonary exacerbation were more favourable in the group receiving antioxidant supplementation (Sagel 2018). Oral supplementation with NAC showed beneficial effects, albeit non-significant, on participants' lung function by preventing the lung function deterioration which was observed in the placebo group. In addition, in a pediatric population (reported by one study), GSH showed a beneficial effect on lung function and nutritional status (Visca 2015).

For inhaled supplements, the meta-analyses are based on four studies, two of which included a relatively large number of participants (258 people with CF) and used similar dosages and inhalation protocols allowing meta analysis based on both studies (Calabrese 2015a; Calabrese 2015b; Griese 2013). Inhalation of GSH or NAC (as a precursor of GSH) into the lungs (the site of inflammation where antioxidants are consumed) of people with cystic fibrosis (CF) resulted in a significantly positive effect on lung function after three months, and the positive effect was maintained also after 9 and 12 months in the adult population in one of the studies.

There is not enough evidence to support the use of the antioxidant supplements reviewed here as a current therapeutical option for improving lung function. However, the administration of NAC, especially by inhalation, seems to prevent a deterioration in lung function and can be considered as a supplementary therapeutic option for people with CF.

Implications for research

As several studies have shown that lung inflammation is present early in infants with CF (Stick 2009), very early supplementation with GSH, either orally as NAC or inhaled, might be an interesting option to explore, especially in countries where neonatal screening is implemented, with the possible use of computer tomography (CT) scans to evaluate the effects on lung inflammation and pathology.

Further work needs to be conducted to clarify the relationship between oxidative stress outcomes and clinically important outcomes; specifically, a rigorous collection of oxidative stress outcomes via systematic review. Whether or not oxidative stress measures are related to clinically important outcomes in CF may increase the efficiency of researching antioxidants in CF and other lung diseases. The introduction of the cystic fibrosis transmembrane regulator (CFTR) modulators in the therapy of an increasing number of people with CF and starting from younger ages might improve the GSH deficiency in the epithelial lung fluid and decrease the oxidative stress and the local inflammation. Investigation of the oxidative stress conditions in people with CF taking CFTR modulators would help answer this question.

An optimal dose and timing of antioxidant supplementation has yet to be determined. In this review, multiple doses were used across studies, making comparisons and grouping based on dose impossible. Similarly, the optimal duration of supplementation would also be worth determining through dose-comparison

studies before further randomised controlled trials are attempted using non-evidence based doses. As data support the hypothesis that the CFTR could be considered as an important actor of antioxidant homeostasis and thus an intrinsic cause of oxidative imbalance in CF airways (Galli 2012), the benefit of antioxidants in people with CF who receive CFTR modulators therapies should also be assessed in the future.

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Original review

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Bishop 2005

Methods	Double-bind, placebo-controlled pilot RCT. Parallel design. Duration: 2 months (8 weeks). Single centre (Utah Valley Regional Medical Center), USA.

Bishop 2005 *(Continued)*

Participants	19 people with CF, 10 randomised to the treatment group and 9 to the placebo group. Gender split: 67% males in treatment group and 60% males in placebo. Age, range: 6 - 19 years.
Interventions	**Treatment**: inhalations with buffered GSH 66 mg/kg distributed across 4 inhalation sessions/day spaced 3 to 4 hours apart. **Control**: placebo.
Outcomes	**Primary outcomes** FEV_1 (% predicted); FVC (% predicted); FEF_{25-75} (% predicted); peak flow (L/min using flow meter). **Secondary outcomes** BMI; 6MWT; sputum colour (self-reported scale) sputum amount (self-reported on scale); sputum viscosity (self-reported on scale); cough frequency (self-reported on scale); general wellness (self-reported on scale); usual stamina (self-reported on scale); improvement (self-reported on scale); measured only at the end of the study.
Funding source	Funding provided by the Utah Valley Institute of Cystic Fibrosis.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Participants were first paired by age and sex and then each member of the pair was randomly assigned to the treatment or placebo groups. No description of how sequence was generated.
Allocation concealment (selection bias)	Low risk	No member of the clinical team was involved in the coding or assignment to treatment or placebo. Non-clinical researchers involved in the study were only provided participant identification number, not participants' names.
Blinding (performance bias and detection bias) oxidative stress	Low risk	The treatment group received capsules containing reduced GSH buffered with sodium bicarbonate, the placebo group received capsules containing sodium chloride with a hint of quinine which was added in order to create a distinct taste and odour. Both participants and the clinical team remained blinded to treatment group assignment throughout the study.
Incomplete outcome data (attrition bias) plasma beta-carotene	Low risk	2 participants discontinued GSH: 1 hospitalised and 1 non-compliant. 3 participants discontinued placebo: 2 hospitalised, 1 non-compliant.
Selective reporting (reporting bias)	Low risk	All outcomes were reported.
Other bias	Unclear risk	Small sample size.

Calabrese 2015 total

Methods	Single-blind, placebo-controlled RCT.

Calabrese 2015 total *(Continued)*

	Parallel design. Duration: 12 months. Multicentre (2 centres) in Italy. Sample size calculated considering as the primary target a 15% change from baseline in % predicted in FEV_1, after 12 months of therapy compared to placebo.
Participants	105 people with CF. Age: 51 children (≥ 6 years and < 18 years), 54 adults (≥ 18 years). Gender: children - 24 girls, 27 boys; adults - 26 females, 28 males.
Interventions	**Treatment**: inhalation with GSH, 2x daily for 12 months. GSH was formulated as vials containing lyophilised powder of reduced GSH to be reconstituted with 8 mL of water, dose 10 mg/kg body weight (max 600 mg). **Control**: placebo - inhaled 0.9% saline (physiological solution) 2x daily for 12 months. Administration of both GSH solution and placebo was through a compressed-air nebulizer (PARI TurboBoy) or an equivalent device delivering particles of 3.5 - 5.5 micrometer in diameter.
Outcomes	**Primary outcome**: 15% change from baseline in % predicted in FEV_1, after 12 months of therapy. **Secondary outcomes**: change from baseline in FVC, FEF_{25-75}, exercise capacity by 6MWT, BMI, BODE index, cough measured by CCIQ, QoL assessed by CFQoL, number of pulmonary exacerbations, antibiotic courses and number of days requiring IV antibiotic treatment compared to the previous year. **Laboratory markers**: CRP at the start and end of study; in a subgroup H_2O_2 in serum and exhaled breath condensate.
Funding source	The study was financed by AIFA (Italian Agency of Drugs) (FARM7K7XZB).
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated randomisation list used. Participants allocated to the pediatric or to the adult group and then to the intervention or placebo.
Allocation concealment (selection bias)	Low risk	Computer-generated randomisation list generated by a person not otherwise involved in the study.
Blinding (performance bias and detection bias) oxidative stress	High risk	Single-blinded because GSH has a distinct taste and smell that is difficult to reproduce as placebo. Participants of the 2 different groups were examined on different days.
Incomplete outcome data (attrition bias) plasma beta-carotene	Low risk	7 from intervention group discontinued (2 children and 5 adults), 1 adult from placebo group discontinued.
Selective reporting (reporting bias)	Low risk	All outcomes are reported.
Other bias	Low risk	None identified.

Calabrese 2015a

Methods	Single-blind, placebo-controlled RCT. Parallel design. Duration: 12 months. Multicentre (2 centres) in Italy. Sample size calculated considering as the primary target a 15% change from baseline in % predicted in FEV_1, after 12 months of therapy compared to placebo.
Participants	105 people with CF in total cohort, 54 adults and 51 children. Age (adults), mean (SD) (range): GSH group 28.9 (9.4) years (19 - 52); placebo group 26 (6.21) years (17 - 41). Gender(adults): - 26 females, 28 males. GSH group 18 males and 13 females; placebo group 10 males and 13 females.
Interventions	**Treatment** (n = 31): inhalation with GSH, 2x daily for 12 months. GSH was formulated as vials containing lyophilised powder of reduced GSH to be reconstituted with 8 mL of water, dose 10 mg/kg body weight (max 600 mg). **Control** (n = 23): placebo - inhaled 0.9% saline (physiological solution) 2x daily for 12 months. Administration of both GSH solution and placebo was through a compressed-air nebulizer (PARI TurboBoy) or an equivalent device delivering particles of 3.5 - 5.5 micrometer in diameter.
Outcomes	**Primary outcome**: 15% change from baseline in % predicted in FEV_1, after 12 months of therapy. **Secondary outcomes**: change from baseline in FVC, FEF_{25-75}, exercise capacity by 6MWT, BMI, BODE index, cough measured by CCIQ, QoL assessed by CFQoL, number of pulmonary exacerbations, antibiotic courses and number of days requiring IV antibiotic treatment compared to the previous year. **Laboratory markers**: CRP at the start and end of study; in a subgroup H_2O_2 in serum and exhaled breath condensate.
Funding source	The study was financed by AIFA (Italian Agency of Drugs) (FARM7K7XZB).
Notes	This study ID is for the adult participants in the study.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated randomisation list used. Participants allocated to the pediatric or to the adult group and then to the intervention or placebo.
Allocation concealment (selection bias)	Low risk	Computer-generated randomisation list generated by a person not otherwise involved in the study.
Blinding (performance bias and detection bias) oxidative stress	High risk	Single-blinded because GSH has a distinct taste and smell that is difficult to reproduce as placebo. Participants of the 2 different groups were examined on different days.
Incomplete outcome data (attrition bias) plasma beta-carotene	Low risk	5 adults from intervention group discontinued, 1 adult from placebo group discontinued.

Calabrese 2015a *(Continued)*

Selective reporting (reporting bias)	Low risk	All outcomes are reported.
Other bias	Low risk	None identified.

Calabrese 2015b

Methods	Single-blind, placebo-controlled RCT. Parallel design. Duration: 12 months. Multicentre (2 centres) in Italy. Sample size calculated considering as the primary target a 15% change from baseline in % predicted in FEV_1, after 12 months of therapy compared to placebo.
Participants	105 people with CF in total cohort, 54 adults and 51 children. Age (children): GSH group 13.37 (3.17) years (6 - 17.9); placebo group 12.29 (3.0) years (7.4 - 16.2). Gender (children):24 girls, 27 boys. GSH group 14 boys and 13 girls; placebo 13 boys and 11 girls.
Interventions	**Treatment** (n = 27): inhalation with GSH, 2x daily for 12 months. GSH was formulated as vials containing lyophilised powder of reduced GSH to be reconstituted with 8 mL of water, dose 10 mg/kg body weight (max 600 mg). **Control** (n = 24): placebo - inhaled 0.9% saline (physiological solution) 2x daily for 12 months. Administration of both GSH solution and placebo was through a compressed-air nebulizer (PARI TurboBoy) or an equivalent device delivering particles of 3.5 - 5.5 micrometer in diameter.
Outcomes	**Primary outcome**: 15% change from baseline in % predicted in FEV_1, after 12 months of therapy. **Secondary outcomes**: change from baseline in FVC, FEF_{25-75}, exercise capacity by 6MWT, BMI, BODE index, cough measured by CCIQ, QoL assessed by CFQoL, number of pulmonary exacerbations, antibiotic courses and number of days requiring IV antibiotic treatment compared to the previous year. **Laboratory markers**: CRP at the start and end of study; in a subgroup H_2O_2 in serum and exhaled breath condensate.
Funding source	The study was financed by AIFA (Italian Agency of Drugs) (FARM7K7XZB).
Notes	This study ID is for the paediatric participants in the study.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated randomisation list used. Participants allocated to the pediatric or to the adult group and then to the intervention or placebo.
Allocation concealment (selection bias)	Low risk	Computer-generated randomisation list generated by a person not otherwise involved in the study.

Calabrese 2015b *(Continued)*

Blinding (performance bias and detection bias) oxidative stress	High risk	Single-blinded because GSH has a distinct taste and smell that is difficult to reproduce as placebo. Participants of the 2 different groups were examined on different days.
Incomplete outcome data (attrition bias) plasma beta-carotene	Low risk	2 children from intervention group discontinued, no children from the placebo group discontinued.
Selective reporting (reporting bias)	Low risk	All outcomes are reported.
Other bias	Low risk	None identified.

Conrad 2015

Methods	Double-blinded, placebo-controlled RCT. Parallel design. Duration: 6 months (24 weeks). Multicentre: 11 centres in the USA. Split into 2 cohorts, a Stanford cohort (n = 16) and a cohort of 54 participants attending 10 other CF centres. Sample size calculations using sputum activity of HNE as primary outcome (70% reduction in activity), as HNE and FEV_1 has shown a strong inverse correlation.
Participants	70 people with CF. Age, range: 7 - 59 years; 25% of cohort were aged 7 - 17 years and the 75% were ≥ 18 years. Gender: overall 50% females (NAC group 44% and placebo group 56%).
Interventions	**Treatment**: 900 mg NAC effervescent tablets 3x daily for 24 weeks. **Control**: identically packed placebo 3x daily for 24 weeks.
Outcomes	**Primary outcome**: log10 human neutrophil elastase activity in sputum. **Secondary outcomes**: lung function measurements, incidence and number of sinus and pulmonary exacerbations, time to first pulmonary or sinus exacerbation, neutrophil count in sputum, concentration of IL-8 in sputum and plasma, concentration of GSH in whole blood, QoL indices.
Funding source	Not specified, but one author is employed by Genetech Inc., where the final manuscript edits were performed.
Notes	4 authors listed as inventors on a provisional patent application covering NAC as a therapeutic agent for CF.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Adaptive randomisation strategy to stratified according to: • baseline FEV_1 % predicted: moderate (FEV_1 between 40% and 60%) versus mild (FEV_1 between 60% and 85%); • age: pediatric (7 - 17 years) versus adult (≥ 18 years);

Conrad 2015 *(Continued)*

		• gender; • and indicators for chronic oral and inhaled antibiotic and chronic ibuprofen use. Randomization assignments were generated by PPD, Inc.
Allocation concealment (selection bias)	Low risk	Randomization assignments and a series of blinded drug kit numbers were generated by PPD, Inc. Kits were distributed to each centre and were assigned with the use of a centralized secure randomisation system at the coordinating centre.
Blinding (performance bias and detection bias) oxidative stress	Low risk	All study personnel and participants were blinded to treatment assignment. The randomisation codes for each participant were revealed to the researchers once recruitment, data collection, and data analyses were completed.
Incomplete outcome data (attrition bias) plasma beta-carotene	Low risk	6 withdrawals in the NAC group (5 participant decision and 1 due to adverse effect not drug related); 2 withdrawals from the control group (1 participant decision and 1 lost to follow-up).
Selective reporting (reporting bias)	Low risk	All outcomes are reported.
Other bias	Unclear risk	2 different cohorts: a Stanford cohort which enrolled participants earlier than the other 10 centres to asses the safety of the treatment for 8 weeks.

Dauletbaev 2009

Methods	Double-blind RCT. Parallel design. Duration: 3-week placebo run-in phase followed by 12 weeks with either high- or low-dose NAC. Single centre in Germany.
Participants	21 people with CF. Mean (range) age: 27.7 (21 - 35) years. Gender: 16 males. FEV_1 > 40% predicted.
Interventions	**Treatment Group 1**: (n = 11) low-dose oral NAC 700 mg/day (1 tablet with 700 mg NAC + 3 placebo tablets). **Treatment Group 2**: (n = 10) oral NAC 2800 mg/day (4 x 700 mg NAC tablets).
Outcomes	FEV_1, extracellular GSH in induced sputum, total leukocyte counts, TNF-α and IL-8 in induced sputum.
Funding source	The study was supported by Hexal AG, Holzkirchen, Germany.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement

Dauletbaev 2009 *(Continued)*

Random sequence generation (selection bias)	Low risk	Participants allocated to either dose of NAC according to a randomisation list (1:1 balanced) generated with Random 1.0 software.
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding (performance bias and detection bias) oxidative stress	Unclear risk	Throughout the study, participants were blinded to taking placebo or active compound or 700 mg/day or 2800 mg/day dosage. The placebo phase was single-blinded, while the NAC treatment phase was double-blinded.
Incomplete outcome data (attrition bias) plasma beta-carotene	Low risk	All randomised participants completed the study and were included in the analysis.
Selective reporting (reporting bias)	Low risk	All outcomes were reported.
Other bias	Low risk	Not identified.

Griese 2013

Methods	Double-blind, placebo-controlled, phase 2b RCT. Parallel design. Duration: 6 months. Multicentre (national) in Germany. Total sample size calculated detect changes in the primary outcome (FEV_1).
Participants	153 people (aged 8 years and older) with CF, 73 randomised to the treatment group and 80 to the placebo group. Age, mean (SD): GSH group 23.08 (9.76) years; placebo group 23.00 (10.38) years. Gender split: GSH group 42/73 (57.5 %) males; placebo group 37/80 (46.3%) males. Other characteristics: FEV_1 40% - 90% predicted.
Interventions	**Treatment**: inhaled GSH 646 mg every 12 hours via eFlow nebulizer. **Control**: placebo every 12 hours via eFlow nebulizer.
Outcomes	**Primary outcomes**: FEV_1 (absolute value and the time-weighted area under the curve of FEV_1 absolute value) change from baseline at end of study (6 months). **Secondary outcomes**: change from baseline in % predicted FEV_1 (at 6 months); time to first pulmonary exacerbation; patient-reported outcomes, as assessed by the CFQoL. **Laboratory markers**: free and total GSH in serum and sputum; inflammatory cells; cytokines; sputum weight.
Funding source	Project supported by grants from the Cystic Fibrosis Foundation (USA), the Mukoviszidose e.V. Germany and the Else-Kroener-Fresenius-Stiftung, Germany.

Griese 2013 *(Continued)*

	PARI Pharma GmbH donated nebuliser devices.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Participants were randomised at a 1:1 ratio by block randomisation within age group. Generation of sequence not described.
Allocation concealment (selection bias)	Low risk	Participants were randomised by central telephone randomisation.
Blinding (performance bias and detection bias) oxidative stress	High risk	Both the test product and the placebo were provided in appropriately covered and identical glass containers to obscure the contents. In addition, identical-looking ampoules for reconstitution of GSH and placebo were provided. However, smell or tastes were not masked due to unresolved toxicology issues of trace agents in long-term usage added to inhalation solutions, therefore the participants receiving GSH could identify it.
Incomplete outcome data (attrition bias) plasma beta-carotene	Low risk	21/73 withdrew from GSH group and 34/80 withdrew from placebo group. The reasons for withdraw in the GSH group were as follows: 9 due to early study termination caused by adverse event (6) and participant request (3); and 12 due to protocol violation caused by lack of FEV_1 values at the inclusion (1) and 11 caused by poor compliance. The reasons for withdraw in the placebo group were as follows: 19 due to early study termination caused by adverse events (5) and participant request (14) and 15 due to protocol violation caused by lack of FEV_1 at the inclusion in the study and 14 due to lack of compliance.
Selective reporting (reporting bias)	High risk	Some of the outcomes (such as GSH levels in sputum, markers of oxidative stress such as protein carbonyls, lipid mediators such as isoprostane) were reported in a subgroup of participants (18 to 24). The GSH levels in sputum neutrophils were reported in 8 participants in each arm.
Other bias	Unclear risk	Oral NAC (a precursor of GSH) was allowed to be continued and these participants could not be identified.

Götz 1980

Methods	Double-blind, placebo-controlled RCT. Cross-over design. Duration: 42 days in total - 3 periods of 14 days. After an initial "washout" period with placebo, either placebo or NAC were given in a random fashion followed by the other substance. Multicenter: 3 centres in Netherlands (Rotterdam), Switzerland (Berne) Austria (Vienna).
Participants	21 children with CF from the pediatric university hospitals in Rotterdam, Berne and Vienna. Age, range: 7.5 to 16 years.

Götz 1980 *(Continued)*

	Gender split: 12 boys, 9 girls.
Interventions	**Treatment**: 9.5 mg/kg NAC in a new galenic form (Fluimucil) 2x daily. **Control**: placebo 2x daily.
Outcomes	Clinical assessment, FVC and FEV_1. No values are presented, just graphical representations.
Funding source	None identified.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described.
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding (performance bias and detection bias) oxidative stress	Low risk	Both substances had a similar taste of orange and were packed in neutral sachets.
Incomplete outcome data (attrition bias) plasma beta-carotene	Unclear risk	Not described.
Selective reporting (reporting bias)	Low risk	All outcomes are reported
Other bias	Low risk	Not identified

Harries 1971

Methods	**Study 1**: 30 children randomly assigned to 1 of 3 treatment arms for period of 1 month. Parallel design. **Study 2**: included 10 children, but not eligible for inclusion in the review since there was no comparator group. Although not clearly stated the study seems to be conducted at the Hospital for Sick Children London, England.
Participants	50 children with CF (diagnostic criteria not stated). 10 children received no vitamin E supplement and served as a control group; 10 received the fat-soluble preparation of vitamin E and 10 children received the water miscible preparation. Gender split: not mentioned. Age: 6 months - 14.5 years. Other characteristics: none had evidence of liver disease and all were treated with moderate reduction in dietary fat together with pancreatic enzymes in the form of Pancrex V.
Interventions	**Study 1**

Harries 1971 *(Continued)*

	Control: 10 children received no vitamin E supplement. **Treatment 1**: 10 children received the fat-soluble preparation in tablet form (Ephynal). **Treatment 2**: 10 children received the water-miscible preparation as a clear water miscible preparation. Both vitamin E preparations given as a single dose of 10 mg/kg per day taken after breakfast. In addition vitamin supplements were given in the form of Abidec (contains no vitamin E).
Outcomes	**Study 1** Serum levels of vitamin E were determined before and at the end of this period and 1 month after discontinuing vitamin E.
Funding source	
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described.
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding (performance bias and detection bias) oxidative stress	High risk	Control group did not receive a placebo but rather no treatment. Moreover, the 2 interventions used were physically different (tablet versus liquid preparations).
Incomplete outcome data (attrition bias) plasma beta-carotene	Unclear risk	A number of children failed to complete the planned period of treatment: serum α-tocopherol levels reported for 9 out of 10 in the fat-soluble vitamin E arm and in 8 out of 10 in the water-soluble vitamin E arm.
Selective reporting (reporting bias)	Low risk	All the outcomes were reported.
Other bias	Unclear risk	Small sample size.

Homnick 1995b

Methods	3-arm RCT, participants stratified by Schwachman score. Parallel design. Duration: 14 months. Single centre in USA.
Participants	20 people with CF diagnosed by sweat test who took regular pancreatic supplements, vitamin supplements (without β-carotene). 10 participants started in the control group and 10 participants in the group taking β-carotene. Gender split: no information available in the paper.

Homnick 1995b *(Continued)*

	Age: over 4 years.
Interventions	**Treatment**: β-carotene 60 mg per day taken in 2x 30 mg doses. Dose was increased individually and periodically during the study in an attempt to obtain plasma concentrations of 0.37 to 0.74 umol/L, believe to be consistent with baseline concentrations in normal persons. Maximum β-carotene dose was 240 mg per day (mean dose among participants 144 mg/day). **Control**: not explicitly stated but assumed to be placebo according to preceding study in same study report.
Outcomes	Plasma β-carotene was measured every 2 weeks for 8 weeks then at least monthly for 12 months.
Funding source	Bronson Clinical Investigation Unit Community Research Fund.
Notes	Multiple dose versus placebo described here. Excluded study Homnick 1995a presents single dose versus placebo.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described.
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding (performance bias and detection bias) oxidative stress	Unclear risk	Control group was not adequately described. Authors do not state whether a placebo was used, or just standard of care.
Incomplete outcome data (attrition bias) plasma beta-carotene	High risk	Out of 20 participants enrolled, 12 completed the study. Of those, 8 were in the control group, 5 on β-carotene. Quote: "No control patient had a significant increase in β-carotene levels throughout the duration of the study." Comment: authors did not present control group data.
Selective reporting (reporting bias)	High risk	Comment: authors claim to take measurements at least monthly for 56 weeks but only report data for baseline and week 50.
Other bias	High risk	Authors do not describe baseline demographics and do not state a sample size calculation. Investigators did not systematically control dose levels throughout the study.

Howatt 1966

Methods	Double-blind, placebo-controlled RCT. Cross-over design with no washout period. Single centre in the USA. Duration: 4x 1-month treatment arms, total study period of 4 months.
Participants	8 people with CF.

Howatt 1966 *(Continued)*

	Age, mean (range): 12.6 (6 - 22) years. Gender split: 3 males, 5 females.
Interventions	**Treatment**: 20% NAC 5 mL nebulized 3x daily. **Control**: placebo (2% NAC) 5 mL nebulized 3x daily.
Outcomes	Physical examination and pulmonary function testing.
Funding source	None identified.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	The order of treatment for the four months period was determined by making two slips of paper for each of the 6 possible combinations and having the participant draw its schedule from an envelope. 1st: 20% NAC / 2% NAC / 20% NAC / 2% NAC. 2nd: 2% NAC / 20% NAC / 2% NAC / 20% NAC. 3rd: 20% NAC / 20% NAC / 2% NAC / 2% NAC [not used]. 4th: 2% NAC / 2% NAC / 20% NAC / 20% NAC. 5th: 2% NAC / 20% NAC / 20% NAC / 2% NAC. 6th: 20% NAC / 2% NAC / 2% NAC / 20% NAC.
Allocation concealment (selection bias)	Low risk	The drugs were supplied in 10 mL vials labelled with a letter code in a sealed envelope which was not opened until the study was completed.
Blinding (performance bias and detection bias) oxidative stress	Low risk	Attempts to mask the odour by using different concentrations of nebulized NAC.
Incomplete outcome data (attrition bias) plasma beta-carotene	Unclear risk	Values of pulmonary function tests only for 2 of the 8 participants.
Selective reporting (reporting bias)	Unclear risk	No data provided.
Other bias	Unclear risk	No washout periods between treatments.

Keljo 2000

Methods	Prospective double-blind, placebo-controlled RCT. Participants stratified according to pulmonary function (70% - 85% predicted and > 85% predicted) and whether or not they used DNase. Parallel design. Duration: 3 months.

Keljo 2000 *(Continued)*

	Single-centre study conducted at the University of Texas Southwestern Medical Center at Dallas, USA. Power considerations based on the primary outcome (cytokine levels) when choosing the number of participants.
Participants	40 people with CF, diagnostic criteria not stated. 19 participants were included in the placebo group and 19 in the α-tocopherol group. 2 of the participants were not included in the analysis. Gender split: male 20 (10 in treatment and 10 in placebo) out of 38 participants. Age: over 6 years old. Other characteristics: mild lung disease (FEV_1 > 70% predicted).
Interventions	**Treatment**: vegetable oil containing naturally occurring RRR-α-tocopherol (dose determined by weight: < 20 kg 600 IU/day, > 20 kg 1200 IU/day). **Control**: vegetable oil containing placebo. All participants took ADEK vitamins for the duration of the study.
Outcomes	Blood tests at beginning and end of study to determine vitamin E levels by HPLC, TNF-α and IL-6 measurement by ELISA. Liver enzymes, PT and PTT taken at end of study.
Funding source	Additional information from authors stated that α-tocopherol and placebo capsules were kindly provided by the Henkel Corporation, 5325 S 9th Ave, LaGrange, IL60525. ADEKs vitamins and partial funding were provided by AXCAN Scandipharm, 22 Inverness Center Parkway, Birmingham, Alabama 35242, USA. It is clear from the results that these did not bias the study. Partial support was also provided by NIH-grant K-24 AT00596.
Notes	Additional information in form of an article not accepted for publication in Pediatric Pulmonology from 2001 was sent by Dr. Keljo.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Described as "randomised" but method not stated.
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding (performance bias and detection bias) oxidative stress	Low risk	Treatment (naturally occurring RRR-α-tocopherol) and placebo both provided in vegetable oil.
Incomplete outcome data (attrition bias) plasma beta-carotene	High risk	There are inconsistencies in the number of participants included in each group between the tables of data reporting and the table describing participant inclusion criteria. Furthermore, data from one subgroup of participants are not reported at all due to the very limited number of participants.
Selective reporting (reporting bias)	High risk	The full paper does not state in the 'Methods' section what the authors planned to report on and the protocol is not available.

Keljo 2000 *(Continued)*

Other bias	Unclear risk	Results have not been published in a peer-reviewed journal; an abstract was presented at the North American Cystic Fibrosis Conference and additional data presented in this review were supplied directly by the authors.

Levin 1961

Methods	Double-blind, placebo-controlled RCT. Parallel design. Duration: 6 months. Single centre: The Fibrocystic Clinic at Babies Hospital, Columbia-Presbyterian Medical Center, New York, USA.
Participants	49 children attending the Fibrocystic Clinic at Babies Hospital (Columbia-Presbyterian Medical Center, New York) randomised. Diagnostic criteria not stated. Paper states "Only patients with a proven diagnosis of cystic fibrosis, and who were apparently stabilized on an accepted regimen of therapy, were accepted for the study." Participants had not previously received supplementary tocopherol. Gender split: in tocopherol group (n = 20) there were 9 males and in the placebo group (n = 25), 17 males. Age: participants were stratified according to age: < 5 years, 5 - 10 years and > 10 years. See note on withdrawals below, for final analysis 45 participants followed for at least 2 months, 37 participants completed 6 months of the study (18 in tocopherol group; 19 in placebo group).
Interventions	**Treatment**: 10 mg/dL α-tocopheryl acetate/kg/day. **Control**: placebo (further details not given). 2 or 3 divided doses of 0.2 mL of mixture/kg/day.
Outcomes	Weight, muscle strength, blood tests (tocopherol level; S-GOT), subjective rating of disease severity (scale of 1 - 5) by outcome assessors, estimate in change of disease status (scale 0 - 6) by outcome assessors after discussions with patients/carers.
Funding source	
Notes	Withdrawals: 3 from placebo group died within the 6 months; 2 declined to continue medication after 2 months; 1 removed from study due to diabetes mellitus; 7 studied for less than 6 months.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	The participants were divided by randomised selection into two similar groups. Randomisation to groups by placing cards labelled '1' or '2' in sealed envelopes in groups of four. Envelopes divided into 3 groups according to age of participants (under 5 years; 5 to 10 years; 10 years and over). No efforts made to counterbalance groups when individuals lost from study.
Allocation concealment (selection bias)	Unclear risk	Envelopes containing allocation in sealed envelopes, but doesn't state if these were opaque or not.
Blinding (performance bias and detection bias)	Low risk	Both preparations had the same taste and were labelled 1 and 2 for identification. Neither the tester nor the participants knew which preparation was being

Levin 1961 *(Continued)*

oxidative stress		taken; blood test done so that examiners could not know the tocopherol levels in participants.
Incomplete outcome data (attrition bias) plasma beta-carotene	High risk	Of 49 participants accepted, 3 died within 6 months, all in the placebo group; 2 others declined to continue the medication after 2 months, 1 participant was removed from the study due to diabetes mellitus, and 7 participants were studied for less than 6 months. In the final analysis there were 45 participants who had been followed for at least 2 months and 37 participants who completed the 6-month period, 18 in the tocopherol group and 19 in the placebo group. Serum tocopherol was reported at 2 and 6 months in 18 and 15, respectively out of 20 participants initially included in the study.
Selective reporting (reporting bias)	Low risk	Serum tocopherol, serum transaminase, weight, subjective improvement and muscle strength are reported in a subgroup of participants at 2 and 6 months.
Other bias	Unclear risk	Small sample size.

Mitchell 1982

Methods	Double-blind, placebo-controlled RCT. Cross-over design (2-week washout period). Duration: 3 months for each treatment arm with 2-week washout in between. Single centre in New Zealand.
Participants	20 children with CF. Age mean (SD): 10.8 (5.9) years. Gender split: 10 males, 10 females.
Interventions	**Treatment**: oral NAC 200 mg 3x daily. **Control**: oral placebo 3x daily.
Outcomes	Clinical assessment, Rx score, PEF rates (the best of 3 was recorded, as performed at home), numerical results provided for weight change, duration of antibiotics and PEF.
Funding source	N-acetylcysteine (Fluimucil® sachets and placebo were supplied by Inpharzam SA, Switzerland).
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomisation process not described.
Allocation concealment (selection bias)	Unclear risk	Not discussed.
Blinding (performance bias and detection bias)	Low risk	Both NAC and placebo were delivered as orange flavoured granules. The code was broken at the end of the study.

Mitchell 1982 *(Continued)*
oxidative stress

Incomplete outcome data (attrition bias) plasma beta-carotene	Unclear risk	4 participants withdrew from the study: 1 due to the development of diabetes mellitus, 2 due to domestic problems and 1 due to the hospitalisation of the mother.
Selective reporting (reporting bias)	Low risk	All data are reported
Other bias	Low risk	Not identified.

Portal 1995a

Methods	RCT. Cross-over design with washout. Duration: 5 months of either treatment - 1 month washout - 5 months alternative treatment. Single centre in France.
Participants	27 people with CF with diagnosis confirmed by 2 positive tests with high sweat electrolytes. Gender split: 12 females, 15 males. Age, range: 7 - 20 years of age. 13 participants (6 girls and 7 boys) underwent the treatment in the following order: selenium/placebo (SP group) and the remaining 14 participants (6 girls and 8 boys) in the reverse order: placebo/selenium (PS group). The washout period was observed for 2 months after the first treatment period.
Interventions	**Treatment**: selenium (sodium selenite) 2.8 µg/kg/day. **Control**: placebo.
Outcomes	Plasma selenium, erythrocyte selenium, GPX-Se, erythrocyte GPX-Se, plasma organic H_2O_2, plasma thiobarbituric acid reactive substances, plasma-induced thiobarbituric acid reactive substances. All measured at 0, 5 and 12 months.
Funding source	Rhone-Alpes region, grant 1999981, the Laurence Foundation and Aguettant Laboratory.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described.
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding (performance bias and detection bias) oxidative stress	Unclear risk	Quote: "double-blind study" Comment: Not otherwise described; insufficient information.

Portal 1995a *(Continued)*

Incomplete outcome data (attrition bias) plasma beta-carotene	High risk	1 participant receiving selenium first who died was excluded from analysis. Only data before the start of the study were available from this participant.
Selective reporting (reporting bias)	Low risk	All intended outcomes were reported.
Other bias	High risk	Authors did not take measurements at baseline before the start of period 2. Data from period 2 not included for meta-analysis since not appropriately measured. Small sample size. The same study appears in full-length manuscripts, published 2 years apart in journals which appear related, but are independent – *Clinical Chemistry* and *Clinica Chimica Acta* (*International Journal of Clinical Chemistry*). Although the 2 reports appear to describe different outcomes of the same study based on their titles (the 1993 paper reports on biological indices of selenium status and the 1995 paper reports on lipid peroxidation markers), the later paper does not reference the methods already reported in the earlier report. Although the earlier report assesses 2 outcomes not later described and the latter report describes two not previously described, there is an overlap of 2 outcomes; neither of which is referred to as having already been reported.

Ratjen 1985

Methods	Double-blind, placebo-controlled RCT. Parallel design. Single centre (Germany). Duration: 2-week washout period prior to starting treatment for 12 weeks.
Participants	36 participants with CF. Age, mean (range): 13.9 (6 - 21) years. Gender split: 16 males, 20 females. Disease status: mild to moderate lung disease. 21 participants included in this Cochrane analysis due to the inclusion criteria of the treatment.
Interventions	3 treatment arms. **Treatment 1**: oral NAC 200 mg 3x daily. **Treatment 2**: oral ambroxol 30 mg 3x daily (not included in the analysis). **Control**: placebo 3x daily.
Outcomes	Lung function including FEV_1 recorded after washout period, at 6 weeks and 12 weeks.
Funding source	Not mentioned.
Notes	

Ratjen 1985 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated randomisation.
Allocation concealment (selection bias)	Unclear risk	Not discussed.
Blinding (performance bias and detection bias) oxidative stress	Low risk	Drugs were given in granular presentation and could not be distinguished with regards to taste, colour and odour.
Incomplete outcome data (attrition bias) plasma beta-carotene	Low risk	4 participants dropped out of the study: 2 due to irregular drug intake, 1 in the placebo group due to missed appointments and 1 due to clinical deterioration.
Selective reporting (reporting bias)	Low risk	All outcomes are reported.
Other bias	Low risk	None identified.

Renner 2001

Methods	Placebo-controlled RCT. Parallel design. Duration: 6 months. Single centre in Austria.
Participants	24 people with CF diagnosed by sweat test; taking regular vitamin supplements and pancreatic enzymes. Gender split: 18 females, 6 males. Age, range: 6.7 years - 27.7 years. 13 participants were included in the group receiving β-carotene and 11 participants in the group receiving placebo.
Interventions	**Intervention**: β-carotene 1 mg/kg/day (max 50 mg/day) for 3 months followed by 10 mg/day for 3 months taken 1x daily. **Control**: placebo.
Outcomes	Lung function (FEV_1 % predicted), plasma β-carotene status and BMI measured at 0 and 6 months. Pulmonary exacerbations and adverse events were also recorded.
Funding source	Funding not stated.
Notes	

Risk of bias

Renner 2001 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Described as randomised, but process not described.
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding (performance bias and detection bias) oxidative stress	Low risk	Quote: "identical appearance". Quote: "the placebo capsules were prepared with starch".
Incomplete outcome data (attrition bias) plasma beta-carotene	Unclear risk	Authors did not describe if there were any withdrawals or dropouts.
Selective reporting (reporting bias)	High risk	Data for nutritional status (BMI) was not completely reported as only baseline values were reported and stated that there was a non-significant effect of supplementation on this outcome. These data cannot be entered into a meta-analysis.
Other bias	High risk	This study suffers from multiple publication (7 different instances - 4 abstracts and 3 full-text reports) and does not refer to previously published studies as such (all stated to be 'original publications'). Small sample size.

Sagel 2018

Methods	Double-blind, placebo-controlled, phase II RCT. Parallel design. Multicentre in USA. Duration: 4 weeks run in and 16 weeks treatment.
Participants	73 people with CF: 36 participants in the AquaADEK-2 capsule group and 37 participants in the control multivitamin group. Age, mean (SD): 22.6 (9.1) years. Gender split: 33 males, 40 females.
Interventions	**Run-in period**: 2 control multivitamin softgel capsules taken orally on a 1x daily basis with pancreatic enzymes and a glass of milk or fat-containing meal for 4 - 8 weeks. **Treatment**: 2 AquADEKs-2* softgel capsules taken orally on a 1x daily basis with pancreatic enzymes and a glass of milk or fat-containing meal for 16 weeks. **Control**: 2 control multivitamin softgel capsules taken orally on a 1x daily basis with pancreatic enzymes and a glass of milk or fat-containing meal for 16 weeks. * AquADEKs-2 containing standard amounts of fat-soluble vitamins (A, D, E, K) as in typical CF multivitamin supplements plus several antioxidants including β-carotene, mixed tocopherols (different forms of vitamin E), CoQ10, mixed carotenoids (lutein, lycopene and zeaxanthin), and the minerals zinc and selenium.

Sagel 2018 *(Continued)*

Outcomes	**Primary outcome**: change in sputum MPO level. **Secondary outcomes**: adverse events; change in systemic antioxidant levels (plasma levels of carotenoids (β-carotene, lutein, zeaxanthin and lycopene), CoQ10, γ-tocopherol, and erythrocyte glutathione peroxidase activity) for absolute values and values corrected for total lipids; change in systemic markers of inflammation and oxidative stress (absolute neutrophil counts, hs-CRP, calprotectin, SAA, MPO, malondialdehyde, protein carbonyls, and total antioxidant capacity measured in plasma; change in 8-iso-PGF2α measured in urine); change in sputum markers of inflammation and oxidative stress (free neutrophil elastase activity, A1AT, SLPI, interleukin-8 (IL-8), TNF-α, 8-iso-PGF2α and 8-Oxo-2'-deoxyguanosine(8-OHdG)); change in systemic vitamin levels (plasma levels of retinol (vitamin A), 25-hydroxy vitamin D, α- tocopherol (vitamin E), and PIVKA-II) for absolute values and values corrected for total lipids; change in lung function (FEV_1 % predicted, FEV_1 (L)), change in growth (weight (kg and z score) and BMI (kg/m^2 and z score)); time to first acute protocol-defined pulmonary exacerbation; number of acute pulmonary exacerbations; number of hospitalizations.
Funding source	No funding acknowledged.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomised 1:1 to receive either the antioxidant-enriched multivitamin ("treated" group) or continue on control multivitamin ("control" group). An adaptive randomisation algorithm was employed based on stratification factors for: age (10 - 17 years, > 18 years), FEV_1 % predicted (40% - 70%, > 70% - 100%), chronic use of inhaled antibiotics, and chronic use of azithromycin.
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding (performance bias and detection bias) oxidative stress	Low risk	The "control" multivitamin and the antioxidant enriched multivitamin ("treated" multivitamin) were identical in appearance and taste.
Incomplete outcome data (attrition bias) plasma beta-carotene	Low risk	2 withdrawals from each group; 35 in the AquADEKs-2 and 30 in the multivitamin control group completed the study. 19 participants were excluded from the per-protocol population: 2 had major protocol violation; 16 had study drug compliance below 80%; 8 had sputum MPO missing at baseline or week 16; and 6 had more than these criteria.
Selective reporting (reporting bias)	Low risk	All data are published.
Other bias	Unclear risk	The complete enrolment of 80 participants was not achieved due to the expiration dates of the antioxidant-enriched and control multivitamins produced for the study.

Stafanger 1988

Methods	Double-blind, placebo-controlled RCT. Cross-over design (no apparent washout period).

Stafanger 1988 *(Continued)*

	Single centre in Denmark. Duration: 6 months treatment in total (3 months on each intervention) followed by 3 months follow-up.
Participants	44 participants with CF, 41 completed study. Age, mean (range): 9.5 (2 - 31 years). Gender split: 23 males, 18 females. No *Pseudomonas aeruginosa* infection.
Interventions	**Treatment**: oral NAC (200 mg 3x daily if under 30 kg or 400 mg 2x daily if over 30 kg). **Control**: placebo tablets contained bicarbonate. Citrus fruits were added to both NAC and placebo.
Outcomes	Subjective symptom scores, pulmonary function tests (presented as differences between NAC and placebo, as it is a cross-over study and divided by year season (administration of NAC during summer or winter)).
Funding source	Study supported by ASTRA A/S , Copenhagen.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No description of randomisation procedure.
Allocation concealment (selection bias)	Unclear risk	Not discussed.
Blinding (performance bias and detection bias) oxidative stress	Unclear risk	Not properly described.
Incomplete outcome data (attrition bias) plasma beta-carotene	High risk	41 participants completed the study out of 44. Lung function reported on 23 participants.
Selective reporting (reporting bias)	High risk	Report of data on 23 participants out of 41.
Other bias	Low risk	Not identified.

Stafanger 1989

Methods	Double-blind, placebo-controlled RCT. Cross-over design with no washout period. Single centre in Denmark. Duration: 2 periods of 3 months receiving either active drug or placebo.

Stafanger 1989 *(Continued)*

Participants	52 participants with CF, 31 (17 males) completing it. Age, mean (range): 15 (7 - 33) years. Gender split (of participants completing study): 17 males, 14 females. Disease status: all chronically infected with *Psuedomonas aeruginosa,* lung function ranged from severely impaired to normal.
Interventions	**Treatment**: oral NAC 200 mg 3x daily (participants under 30 kg) or oral NAC 400 mg 2x daily (participants over 30 kg). **Control**: placebo (bicarbonate tablets).
Outcomes	Subjective scores, pulmonary function tests including FVC and FEV_1.
Funding source	The study was supported by ASTRA A/S, Copenhagen.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomization process not described.
Allocation concealment (selection bias)	Unclear risk	Not discussed.
Blinding (performance bias and detection bias) oxidative stress	Unclear risk	No description.
Incomplete outcome data (attrition bias) plasma beta-carotene	Unclear risk	21 participants were excluded, 10 because of poor cooperation and the rest due to development of: Quincke edema (n = 1), exantema (n = 1), abdominal pain (n = 2), increase of non-producing coughing (n = 1), disliked the taste (n = 2), exacerbation (n = 1) and change in antibiotic treatment (n = 2).
Selective reporting (reporting bias)	High risk	FEV_1 reported for 10 out of 31 participants, only in those with baseline peak expiratory flow less than 70% of predicted for sex, age and height.
Other bias	Low risk	Not identified.

Visca 2015

Methods	Double-blind, placebo-controlled RCT. Parallel design. Duration: 6 months. Undertook sample size calculation which stated a minimum number of participants would be 22 in each group to allow detection of an effect size of 8% (weight percentile).
Participants	47 children over 18 months of age with CF diagnosed by sweat test or genetically.

Visca 2015 *(Continued)*

	GSH group n = 24; placebo group n = 23. Age, mean (SD): GSH group 67.3 (29.8) months; placebo group 66.9 (32.4) months. Gender split: GSH group 14 females and 10 males; placebo group 10 females and 13 males.
Interventions	**Treatment**: oral reduced GSH 65 mg/kg, divided into 3 doses/day. **Control**: placebo (calcium citrate) 65 mg/kg divided into 3 doses/day.
Outcomes	**Primary outcomes**: change in weight percentile; BMI percentile; height percentile; fecal calprotectin. **Secondary outcomes**: white blood cell count (1000/mm3), ALT (U/L), vitamin E level (mg/mL?), CRP (mg/L). Pulmonary function tests (FEV_1 % predicted, FVC % predicted) were reported only as poster results.
Funding source	Support from Flatley Foundation and PACFI.
Notes	Participants were recruited through an Internet CF group in Italy. Additional information was obtained from one of the co-authors, Clark Bishop.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Age-stratified: a young group 18 months - 3 years; and an older group aged > 3 years. Randomly assigned to treatment or placebo by use of a random-number generator.
Allocation concealment (selection bias)	Low risk	The containers were labelled 'A' or 'B' by the pharmaceutical supplier and the blind was removed only after the study had concluded and data analysis begun.
Blinding (performance bias and detection bias) oxidative stress	Low risk	Described as double-blinded, placebo and GSH materials were encapsulated and identical in appearance. The containers were labelled 'A' or 'B' by the pharmaceutical supplier, thus blinding the study to physician, the clinic staff, and the research study team to their contents. Participants were also blind to the treatment they were receiving.
Incomplete outcome data (attrition bias) plasma beta-carotene	Low risk	Excluded from analysis: 2 in GSH group (1 did not arrived at visit at 6 months and 1 did not followed study protocol and discontinued study) and 1 in placebo group (due to worsening of the clinical status). Many missing values when the raw spirometry data were investigated.
Selective reporting (reporting bias)	High risk	Authors report only some outcomes in the published article. The lung function measurements are analysed and presented only in the poster results.
Other bias	Unclear risk	Because of recruitment logistics, there were 4 possible start dates for the study participants: March, May, July, September. Difference in between groups in numbers of participants who were delF508 homozygotes (usually have a more severe disease than other mutations): GSH group 13.6%; placebo group 27.7%. Small sample size.

Wood 2003

Methods	RCT. Parallel design. Duration: 8 weeks. Single centre (children's hospital) in Australia.
Participants	46 children > 5 years of age with CF (diagnosis confirmed by sweat test) completed study. Group A (low-dose supplement) n = 24 participants; Group B (high-dose supplement) n = 22 participants. Age, mean (SEM): Group A 10.6 (0.7) years; Group B 12.6 (0.8) years. Gender split: Group A 8 males and 16 females; Group B 13 males and 9 females.
Interventions	All participants discontinued vitamin supplementation prior to enrolment but were supplemented with vitamin E and A for 4 weeks before study start. **Treatment**: combined supplement (200 mg vitamin E (RRR a-tocopherol), 300 vitamin C (sodium ascorbate), 25 mg β-carotene, 90 µg selenium (selenomethionine), 500 µg vitamin A (retinyl palmitate in oil)) once per day with breakfast. **Control**: continuation of low-dose supplement (10 mg vitamin E + 500 µg vitamin A) taken for 4 weeks prior to study start.
Outcomes	Lung function (FEV_1 % predicted), quality of well-being, lipid peroxidation, plasma antioxidant status, plasma fatty acid status, pulmonary exacerbations measured at 0 and 8 weeks.
Funding source	Research Management Committee grant from University of Newcastle.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "derived using a random-numbers computer program".
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding (performance bias and detection bias) oxidative stress	Unclear risk	Not described.
Incomplete outcome data (attrition bias) plasma beta-carotene	Unclear risk	Authors did not state initial enrolment numbers and it is unclear whether or not participant data is missing.
Selective reporting (reporting bias)	Low risk	Authors report all outcomes as stated.
Other bias	Unclear risk	Small sample size.

6MWT: 6-minute walking test
ALT: alanine transaminase
BMI: body mass index
BODE index: body mass index, airflow obstruction, dyspnoea, exercise capacity
CCIQ: chronic cough impact questionnaire
CF: cystic fibrosis
CFQoL: Cystic Fibrosis Quality of Life Questionnaire
CoQ10: co-enzyme Q10
CRP: C-reactive protein
DNase: dornase alfa
FEV_1: forced expiratory volume at one second
FVC: forced vital capacity
FEF_{25-75}: forced expiratory flow at between 25% and 75% of forced vital capacity
GPX-Se: plasma selenium dependent glutathione peroxidise
GSH: glutathione
H_2O_2: hydrogen peroxide
HNE: human neutrophil elastase
HPLC: high performance liquid chromatography
IgG: immunoglobulin G
IV: intravenous
MPO: myeloperoxidase
NAC: N-acetylcysteine
PEF: peak expiratory flow
PT: prothrombin time
PTT: partial thromboplastin time
QoL: quality of life
RCT: randomised controlled trial
Rx: treatment
SEM: standard error of the mean
S-GOT: serum glutamic-oxaloacetic transaminase

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Abdulhamid 2008	No relevant intervention.
Anonymous 1975	Review article.
Beddoes 1981	Review article.
Best 2004	No relevant intervention.
Bines 2005	Prospective cohort study.
Cobanoglu 2002	Case-control study.
Congden 1981	Case-control study.
Ekvall 1978	Prospective cohort study.
Farrell 1977	Case-control study.
Goodchild 1986	Review article.
Homnick 1995a	Single-dose study.
Hoogenraad 1989	Case report.

Study	Reason for exclusion
Hubbard 1980	Case report.
Jacquemin 2009	Single dose administration of vitamin E to test bioavailability of 2 different formulations.
Kauf 1995	Prospective cohort study.
Kawchak 1999	Prospective cohort study.
Kelleher 1987	Prospective cohort study.
Khorasani 2009	Intervention not eligible - study of zinc supplementation.
Knopfle 1975	Case-control study.
Lancellotti 1996	Case-control study.
Lepage 1996	Case-control study.
Madarasi 2000	Case-control study.
Mischler 1991	RCT - not pre-specified antioxidant intervention.
Munck 2010	Prospective cohort study, no controls.
Nasr 1993	RCT - active control arm (equivalency trial).
Oermann 2001	Review article.
Oudshoorn 2007	RCT - multiple micronutrients including some of the included interventions.
Papas 2007	RCT - active control arm (equivalency trial).
Peters 1996	RCT - active control arm (equivalency trial).
Portal 1995b	Case-control study.
Powell 2010	No relevant intervention.
Rawal 1974	Prospective cohort study.
Rettammel 1995	Prospective cohort study.
Richard 1990	Two studies: case control and prospective cohort.
Sharma 2016	Intervention not eligible - study of zinc supplementation.
Sokol 1989	Prospective cohort study.
Sung 1980	Prospective cohort study.
Uden 1990	Participant population: chronic pancreatitis.
Underwood 1972a	Retrospective cohort study.
Underwood 1972b	Case-control study.

Study	Reason for exclusion
van der Vliet 1997	Review article.
Winklhofer-Roob 1995	Case-control study.
Winklhofer-Roob 1996a	Letter to the editor.
Winklhofer-Roob 1996b	RCT - active control (non-inferiority trial).
Winklhofer-Roob 1996c	Case-control study.
Winklhofer-Roob 1997a	Case-control study.
Winklhofer-Roob 1997b	Letter to the editor.
Winklhofer-Roob 1997c	Letter to the editor.
Winklhofer-Roob 2003	Review article.
Wojewodka 2015	Intervention not eligible - fenretinide is a synthetic retinoid derivative and not an antioxidant supplement.
Wood 2002	Prospective cohort study.
Zoirova 1983	Review article (Russian).

GSH: glutathione
RCT: randomised controlled trial

Characteristics of studies awaiting assessment *[ordered by study ID]*

Tirouvanziam 2005

Methods	RCT. Phase I. Duration: 4 weeks
Participants	Exclusion criteria: inability to undergo sputum induction (aged under 11 years and bronchial reactivity), FEV_1 % predicted below 40%, intake of any antioxidant in the 4 weeks leading up to study. 18 participants with CF randomised to 1 of 3 cohorts.
Interventions	Cohort 1: NAC 1.8 g/day, 3 times daily. Cohort 2: NAC 2.4 g/day, 3 times daily. Cohort 3: NAC 3.0 g/day, 3 times daily.
Outcomes	Adverse effects, CF QoL, complete blood count and chemistry, whole blood GSH (measured by HPLC), intracellular GSH (measured by FACS), sputum analysis, compliance.
Notes	Study supported by BioAdvantex, Inc. and Cystic Fibrosis Foundation Therapeutics, Inc.

Tirouvanziam 2006

Methods	Double-blind, placebo-controlled RCT. Phase 2. Duration: 12 weeks.
Participants	Exclusion criteria: inability to undergo sputum induction (aged under 8 years and bronchial reactivity), FEV_1 % predicted below 40%, intake of any antioxidant in the 4 weeks leading up to study. 21 participants with CF.
Interventions	Intervention: NAC 2.7 g/day, 3 times daily. Control: placebo.
Outcomes	LIve neutrophil count in sputum, pulmonary function tests (FEV_1 % predicted), adverse events, CF QoL, complete blood count, serum chemistries, intracellular GSH (measured by FACS), sputum analysis.
Notes	Followed by a further 12-week open-label drug-only phase. Study supported by BioAdvantex, Inc. and Cystic Fibrosis Foundation Therapeutics, Inc.

Wong 1988

Methods	Participants split into 3 groups (method of randomisation not specified). Parallel design. Duration: 10 - 14 days.
Participants	30 people with CF admitted for pulmonary exacerbations - diagnostic criteria not stated.
Interventions	Group A: oral fat-soluble vitamin E 10 mg/kg/day. Group B: oral water-miscible vitamin E (Aquasol E) 10 mg/kg/day. Group C: no supplementation.
Outcomes	Serum for vitamin E levels at beginning and end of treatment analysed by HPLC; 3 day fecal fat excretion.
Notes	Abstract only. Participants received appropriate intravenous antibiotics together with daily infusion of 10% Nutralipid 15 ml/kg/day. Also continued to receive enteric coated pancreatic enzymes in usual dosage.

CF: cystic fibrosis
FACS: fluorescence-activated cell sorting
FEV_1: forced expiratory volume at one second
HPLC: high performance liquid chromatography
NAC: N-acetylcysteine
QoL: quality of life

DATA AND ANALYSES

Comparison 1. Oral antioxidants versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Lung function: FEV_1 (% predicted) (mean change from baseline)	8		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.1 At 2 months (combined supplement)	1	46	Mean Difference (IV, Random, 95% CI)	-4.3 [-5.64, -2.96]
1.2 At 3 months (NAC)	4	125	Mean Difference (IV, Random, 95% CI)	2.83 [-2.16, 7.83]
1.3 at 4 months (multivitamins with antioxidants versus multivitamin alone	1	69	Mean Difference (IV, Random, 95% CI)	1.44 [-2.23, 5.11]
1.4 At 6 months (NAC)	1	62	Mean Difference (IV, Random, 95% CI)	4.38 [0.89, 7.87]
1.5 At 6 months (GSH)	1	47	Mean Difference (IV, Random, 95% CI)	17.4 [13.97, 20.83]
1.6 At 6 months (β-carotene)	1	24	Mean Difference (IV, Random, 95% CI)	0.9 [-20.09, 21.89]
2 Lung function: FVC (% predicted) mean change from baseline	5		Mean Difference (IV, Random, 95% CI)	Subtotals only
2.1 At 2 months (combined supplement)	1	46	Mean Difference (IV, Random, 95% CI)	-4.2 [-11.28, 2.88]
2.2 At 3 months (NAC)	3	115	Mean Difference (IV, Random, 95% CI)	3.34 [-4.30, 10.97]
2.3 At 6 months (NAC)	1	62	Mean Difference (IV, Random, 95% CI)	3.75 [-0.13, 7.63]
2.4 At 6 months (GSH)	1	47	Mean Difference (IV, Random, 95% CI)	14.80 [10.07, 19.53]
3 QoL: Quality of Well Being Scale	2		Std. Mean Difference (IV, Random, 95% CI)	Subtotals only
3.1 At 2 months (combined supplement)	1	46	Std. Mean Difference (IV, Random, 95% CI)	-0.66 [-1.26, -0.07]
3.2 at 3 months (NAC) CF-Q respiratory domain scale	1	62	Std. Mean Difference (IV, Random, 95% CI)	0.33 [-0.17, 0.83]
3.3 at 6 months (NAC) CF-Q respiratory domain scale	1	61	Std. Mean Difference (IV, Random, 95% CI)	-0.03 [-0.53, 0.47]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4 Oxidative stress: lipid peroxidation (H_2O_2) (µmol/L)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
4.1 At 5 months (selenium)	1	27	Mean Difference (IV, Random, 95% CI)	15.90 [-13.16, 44.96]
5 Oxidative stress: lipid peroxidation (F_2-isoprostanes) (ng/L)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
5.1 At 2 months (combined supplement)	1	46	Mean Difference (IV, Random, 95% CI)	1.0 [-23.94, 25.94]
6 Oxidative stress: lipid peroxidation (malondialdehyde) (µmol/L) mea difference to baseline)	2	51	Mean Difference (IV, Random, 95% CI)	-0.10 [-0.45, 0.25]
6.1 At 5 months (selenium)	1	27	Mean Difference (IV, Random, 95% CI)	-0.26 [-0.67, 0.15]
6.2 At 6 months (β-carotene)	1	24	Mean Difference (IV, Random, 95% CI)	0.10 [-0.38, 0.58]
7 Oxidative stress: urine 8-iso-PGF2α log 10 (pg/mL)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
7.1 At 4 months	1	69	Mean Difference (IV, Random, 95% CI)	0.09 [-0.10, 0.28]
8 Oxidative stress: sputum 8-iso-PGF2α log10 (pg/mL)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
8.1 at 4 months	1	65	Mean Difference (IV, Random, 95% CI)	0.02 [-0.12, 0.16]
9 Oxidative stress: sputum 8-OHdG (log10) ng/ml	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
9.1 At 4 months	1	65	Mean Difference (IV, Random, 95% CI)	-0.07 [-0.24, 0.10]
10 Oxidative stress: enzyme function - GPX (U/g Hb)	2	73	Mean Difference (IV, Random, 95% CI)	4.96 [-3.26, 13.19]
10.1 At 2 months (combined supplement)	1	46	Mean Difference (IV, Random, 95% CI)	1.6 [0.30, 2.90]
10.2 At 5 months (selenium)	1	27	Mean Difference (IV, Random, 95% CI)	10.20 [2.22, 18.18]
11 Oxidative stress: enzyme function - SOD (U/mg Hb)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
11.1 At 2 months (combined supplement)	1	46	Mean Difference (IV, Random, 95% CI)	0.27 [-1.24, 1.78]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
12 Oxidative stress: potency (TEAC) (mmol/L)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
12.1 At 6 months (β-carotene)	1	24	Mean Difference (IV, Random, 95% CI)	0.04 [-0.17, 0.25]
13 Plasma total antioxidant capacity (nmol) (change from baseline)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
13.1 At 3 months (β-carotene)	1	24	Mean Difference (IV, Random, 95% CI)	0.1 [-0.18, 0.38]
13.2 At 6 months (β-carotene)	1	24	Mean Difference (IV, Random, 95% CI)	0.04 [-0.30, 0.38]
14 Plasma total antioxidant capacity (log10) (CRE)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
14.1 At 1 month	1	66	Mean Difference (IV, Random, 95% CI)	0.0 [-0.02, 0.02]
14.2 At 4 months	1	72	Mean Difference (IV, Random, 95% CI)	-0.01 [-0.04, 0.02]
15 Plasma antioxidant status: vitamin E (µmol/L)	5		Mean Difference (IV, Random, 95% CI)	Subtotals only
15.1 At 1 month (water-miscible vitamin E)	1	18	Mean Difference (IV, Random, 95% CI)	26.7 [15.90, 37.50]
15.2 At 1 month (fat-soluble vitamin E)	1	19	Mean Difference (IV, Random, 95% CI)	13.47 [9.05, 17.89]
15.3 At 1 month (antioxidant-enriched multivitamin)	1	66	Mean Difference (IV, Random, 95% CI)	-3.48 [-8.01, 1.05]
15.4 At 2 months (water-miscible vitamin E and combined supplement	2	83	Mean Difference (IV, Random, 95% CI)	10.65 [6.53, 14.77]
15.5 at 4 month (antioxidant -enriched multivitamin)	1	72	Mean Difference (IV, Random, 95% CI)	-1.86 [-6.36, 2.64]
15.6 At 6 months (water-miscible vitamin E)	1	33	Mean Difference (IV, Random, 95% CI)	19.73 [12.48, 26.98]
15.7 At 6 months (oral GSH)	1	44	Mean Difference (IV, Random, 95% CI)	4.26 [2.03, 6.49]
16 Plasma antioxidant status: β-carotene (µmol/L)	2	70	Mean Difference (IV, Random, 95% CI)	0.13 [0.02, 0.25]
16.1 At 2 months (combined supplement)	1	46	Mean Difference (IV, Random, 95% CI)	0.1 [0.02, 0.18]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
16.2 At 6 months (β-carotene)	1	24	Mean Difference (IV, Random, 95% CI)	0.24 [0.02, 0.46]
17 Plasma antioxidant status: selenium (µmol/L)	2	73	Mean Difference (IV, Random, 95% CI)	0.48 [0.27, 0.68]
17.1 At 2 months (combined supplement)	1	46	Mean Difference (IV, Random, 95% CI)	0.6 [0.39, 0.81]
17.2 At 5 months (selenium)	1	27	Mean Difference (IV, Random, 95% CI)	0.39 [0.27, 0.51]
18 Plasma antioxidant status: vitamin C (µmol/L)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
18.1 At 2 months (combined supplement)	1	46	Mean Difference (IV, Random, 95% CI)	8.0 [-15.05, 31.05]
19 Plasma antioxidant status: whole blood GSH (µmol/L) (change from baseline)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
19.1 at 3 months	1	61	Mean Difference (IV, Random, 95% CI)	19.00 [-183.58, 221.58]
19.2 at 6 months	1	60	Mean Difference (IV, Random, 95% CI)	64.10 [-170.05, 298.25]
20 Plasma antioxidant status: plasma fatty acid status (mg/L)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
20.1 At 2 months (combined supplement)	1	46	Mean Difference (IV, Random, 95% CI)	166.0 [-61.38, 393.38]
21 Plasma inflammation: IL-6 (pg/mL) at 3 months (vitamin E)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
21.1 FEV_1 > 85% and no DNase	1	12	Mean Difference (IV, Random, 95% CI)	-2.02 [-4.63, 0.59]
21.2 FEV_1 > 85% and DNase	1	9	Mean Difference (IV, Random, 95% CI)	-0.31 [-4.03, 3.41]
21.3 FEV_1 range 70% - 85% and DNase	1	11	Mean Difference (IV, Random, 95% CI)	-0.24 [-3.80, 3.32]
22 Plasma inflammation: TNF-α (pg/mL) at 3 months (vitamin E)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
22.1 FEV_1 > 85% and no DNase	1	12	Mean Difference (IV, Random, 95% CI)	-1.37 [-3.61, 0.87]
22.2 FEV_1 > 85% and DNase	1	14	Mean Difference (IV, Random, 95% CI)	0.33 [-0.49, 1.15]

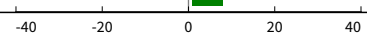
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
22.3 FEV_1 range 70% - 85% and DNase	1	11	Mean Difference (IV, Random, 95% CI)	-0.94 [-1.61, -0.26]
23 Plasma IL-8 pg/mL (log 10) (change from baseline)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
23.1 At 3 months	1	61	Mean Difference (IV, Random, 95% CI)	0.01 [-0.19, 0.21]
23.2 At 6 months	1	56	Mean Difference (IV, Random, 95% CI)	-0.09 [-0.32, 0.14]
24 Sputum IL-8 pg/ml (log 10) (per volume)	3		Mean Difference (IV, Random, 95% CI)	Subtotals only
24.1 At 3 months (NAC)	2	92	Mean Difference (IV, Random, 95% CI)	-0.01 [-0.15, 0.14]
24.2 At 4 months (antioxidant enriched vitamins)	1	65	Mean Difference (IV, Random, 95% CI)	-0.06 [-0.24, 0.12]
24.3 At 6 months (NAC)	1	56	Mean Difference (IV, Random, 95% CI)	0.19 [-0.03, 0.41]
25 Sputum human neutrophil elastase (log 10) (mg/mg) per weight (change from baseline)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
25.1 At 3 months	1	61	Mean Difference (IV, Random, 95% CI)	-0.04 [-0.24, 0.16]
25.2 At 6 months	1	56	Mean Difference (IV, Random, 95% CI)	0.11 [-0.11, 0.33]
26 Sputum neutrophil count (logarithm)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
26.1 at 3 months	1	63	Mean Difference (IV, Random, 95% CI)	1.90 [-8.08, 11.88]
26.2 at 6 months	1	61	Mean Difference (IV, Random, 95% CI)	2.60 [-11.85, 17.05]
27 Sputum myeloperoxidase (MPO) levels (log 10) (ng/mL)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
27.1 At 4 months (multivitamins with antioxidants versus multivitamin alone	1	65	Mean Difference (IV, Random, 95% CI)	-0.13 [-0.48, 0.22]
28 Nutritional status: BMI (change from baseline)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
28.1 At 3 months (NAC)	1	64	Mean Difference (IV, Random, 95% CI)	0.30 [-0.02, 0.62]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
28.2 At 6 months (NAC)	1	62	Mean Difference (IV, Random, 95% CI)	0.2 [-0.23, 0.63]
29 Nutritional status: BMI percentile (change from baseline)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
29.1 At 3 months (GSH)	1	40	Mean Difference (IV, Random, 95% CI)	9.2 [6.22, 12.18]
29.2 At 6 months (GSH)	1	40	Mean Difference (IV, Random, 95% CI)	17.20 [14.35, 20.05]
30 Nutritional status: weight (kg) (change from baseline)	3		Mean Difference (IV, Random, 95% CI)	Subtotals only
30.1 At 3 months (NAC)	2	84	Mean Difference (IV, Random, 95% CI)	0.24 [-0.73, 1.22]
30.2 At 6 months (NAC)	1	62	Mean Difference (IV, Random, 95% CI)	0.60 [-0.51, 1.71]
30.3 At 6 months (water-miscible vitamin E)	1	36	Mean Difference (IV, Random, 95% CI)	-0.30 [-7.19, 6.59]
31 Nutritional status: weight percentile (change from baseline)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
31.1 At 3 months (GSH)	1	44	Mean Difference (IV, Random, 95% CI)	8.1 [5.64, 10.56]
31.2 At 6 months (GSH)	1	44	Mean Difference (IV, Random, 95% CI)	17.0 [14.64, 19.36]
32 Antibiotic days per participant	2	70	Mean Difference (IV, Random, 95% CI)	-4.28 [-15.16, 6.60]
32.1 At 2 months (combined supplement)	1	46	Mean Difference (IV, Random, 95% CI)	4.0 [-14.06, 22.06]
32.2 At 6 months (β-carotene)	1	24	Mean Difference (IV, Random, 95% CI)	-8.0 [-18.78, 2.78]
33 Number of participants with at least one exacerbation	2	143	Risk Ratio (M-H, Random, 95% CI)	0.80 [0.59, 1.09]
33.1 At 4 months (multivitamins with antioxidants versus multivitamin alone	1	73	Risk Ratio (M-H, Random, 95% CI)	0.78 [0.53, 1.14]
33.2 At 6 months (NAC)	1	70	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.50, 1.39]
34 Number of hospitalizations	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only

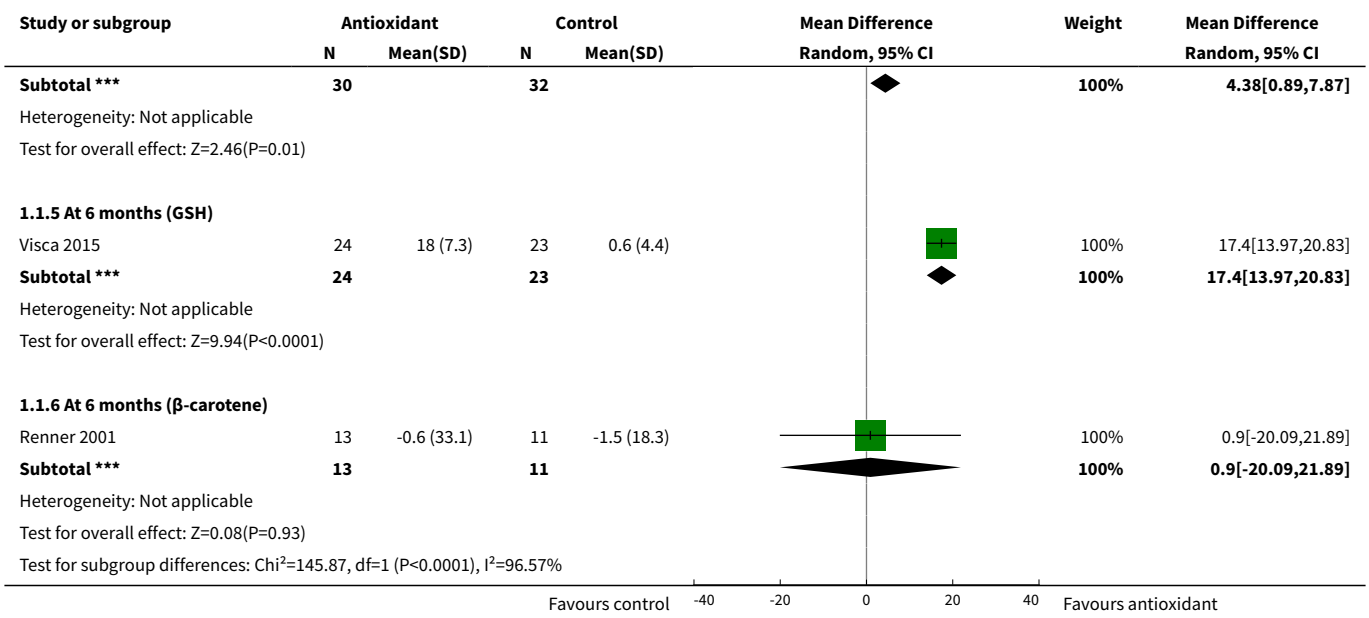
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
34.1 At 6 months (NAC)	1	70	Risk Ratio (M-H, Random, 95% CI)	0.94 [0.49, 1.81]
35 Adverse effects	3		Odds Ratio (M-H, Random, 95% CI)	Subtotals only
35.1 Sinusitis	3	181	Odds Ratio (M-H, Random, 95% CI)	1.58 [0.38, 6.55]
35.2 Distal intestinal obstruction syndrome	3	181	Odds Ratio (M-H, Random, 95% CI)	0.47 [0.09, 2.34]
35.3 Diahorrea	3	181	Odds Ratio (M-H, Random, 95% CI)	1.76 [0.58, 5.32]
35.4 Pulmonary exacerbations	2	111	Odds Ratio (M-H, Random, 95% CI)	0.57 [0.23, 1.41]
35.5 Elevated liver enzymes (ALT)	2	108	Odds Ratio (M-H, Random, 95% CI)	3.04 [0.31, 30.19]

Analysis 1.1. Comparison 1 Oral antioxidants versus control, Outcome 1 Lung function: FEV_1 (% predicted) (mean change from baseline).

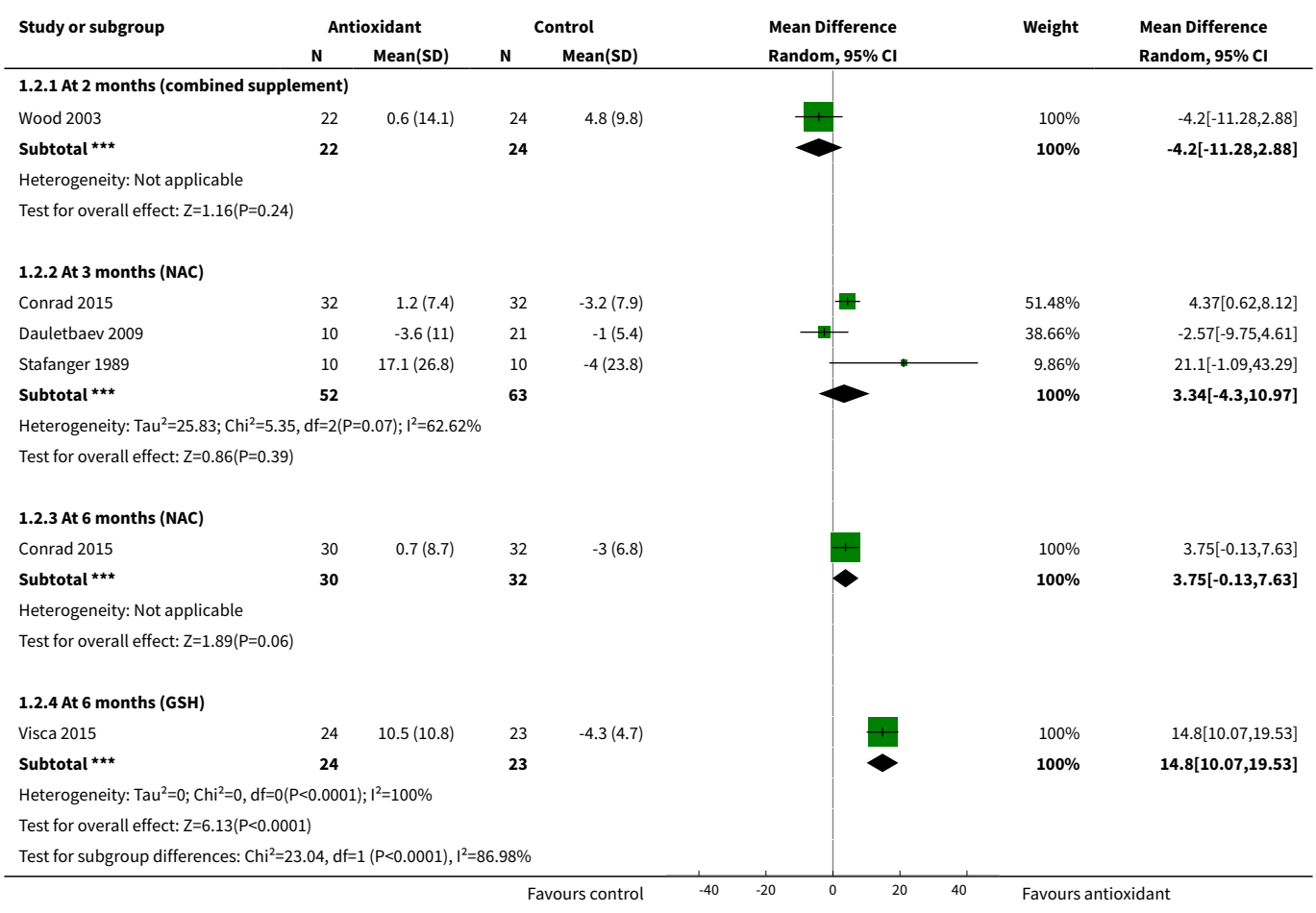
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.1.1 At 2 months (combined supplement)							
Wood 2003	22	-3 (2.7)	24	1.3 (1.8)		100%	-4.3[-5.64,-2.96]
Subtotal ***	**22**		**24**			**100%**	**-4.3[-5.64,-2.96]**
Heterogeneity: Not applicable							
Test for overall effect: Z=6.3(P<0.0001)							
1.1.2 At 3 months (NAC)							
Conrad 2015	32	-0 (6.1)	32	-2.6 (6.6)		44.37%	2.59[-0.53,5.71]
Dauletbaev 2009	10	-4.7 (10.2)	10	-1.6 (5.5)		25.53%	-3.04[-10.2,4.12]
Ratjen 1985	10	-3.6 (9.8)	11	-8.6 (8.5)		23.02%	5[-2.87,12.87]
Stafanger 1989	10	12.6 (17.5)	10	-5.9 (22.2)		7.08%	18.5[1.01,35.99]
Subtotal ***	**62**		**63**			**100%**	**2.83[-2.16,7.83]**
Heterogeneity: Tau^2=12.09; Chi^2=5.9, df=3(P=0.12); I^2=49.19%							
Test for overall effect: Z=1.11(P=0.27)							
1.1.3 at 4 months (multivitamins with antioxidants versus multivitamin alone							
Sagel 2018	34	-0.8 (8)	35	-2.2 (7.5)		100%	1.44[-2.23,5.11]
Subtotal ***	**34**		**35**			**100%**	**1.44[-2.23,5.11]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.77(P=0.44)							
1.1.4 At 6 months (NAC)							
Conrad 2015	30	0.9 (7.7)	32	-3.5 (6.2)		100%	4.38[0.89,7.87]



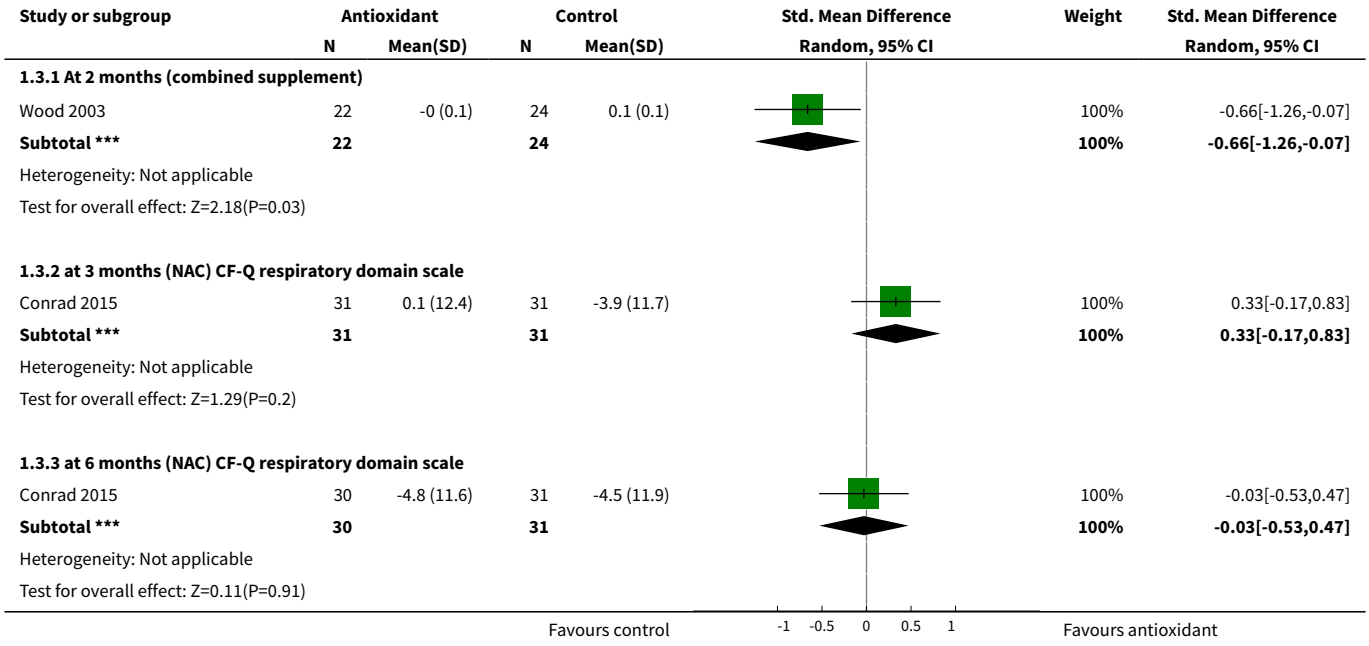
Favours control — Favours antioxidant



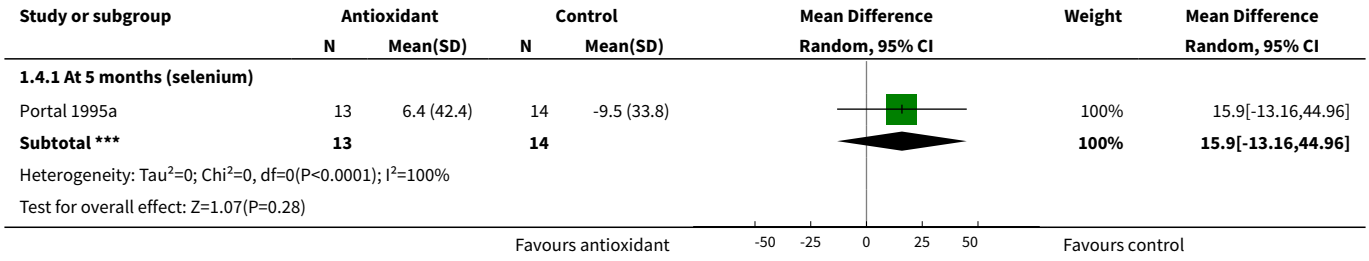
Analysis 1.2. Comparison 1 Oral antioxidants versus control, Outcome 2 Lung function: FVC (% predicted) mean change from baseline.



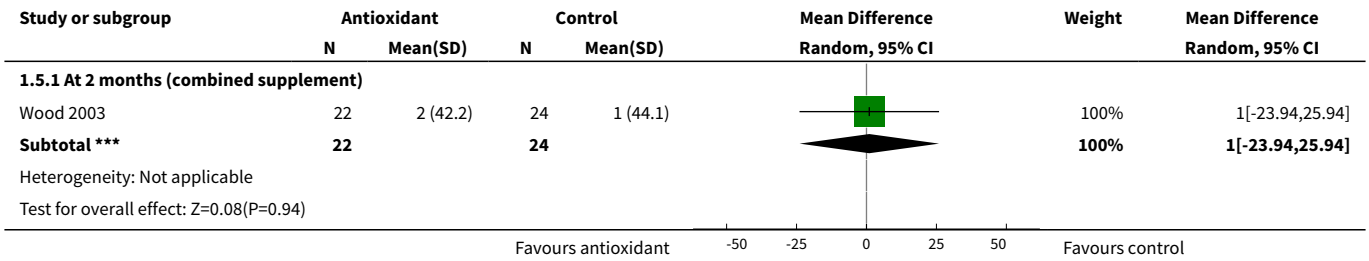
Analysis 1.3. Comparison 1 Oral antioxidants versus control, Outcome 3 QoL: Quality of Well Being Scale.



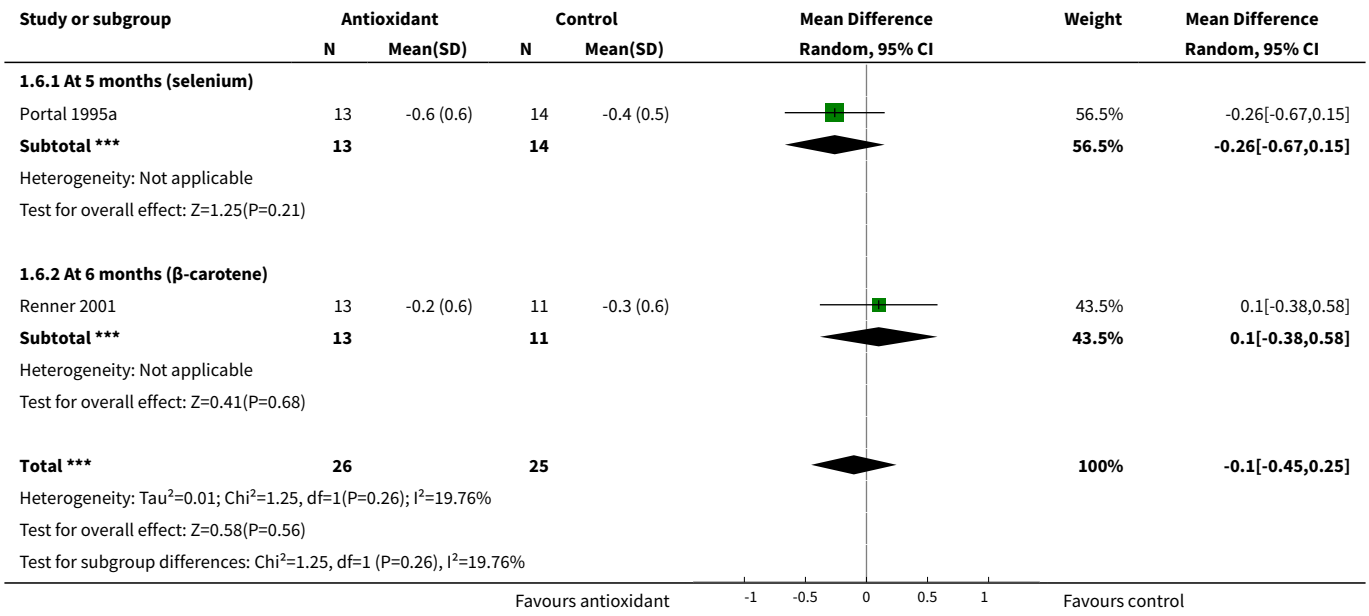
Analysis 1.4. Comparison 1 Oral antioxidants versus control, Outcome 4 Oxidative stress: lipid peroxidation (H_2O_2) (µmol/L).



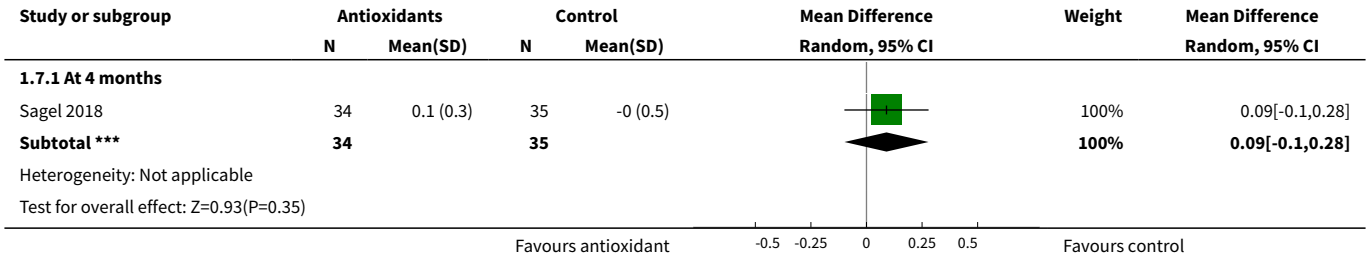
Analysis 1.5. Comparison 1 Oral antioxidants versus control, Outcome 5 Oxidative stress: lipid peroxidation (F_2-isoprostanes) (ng/L).



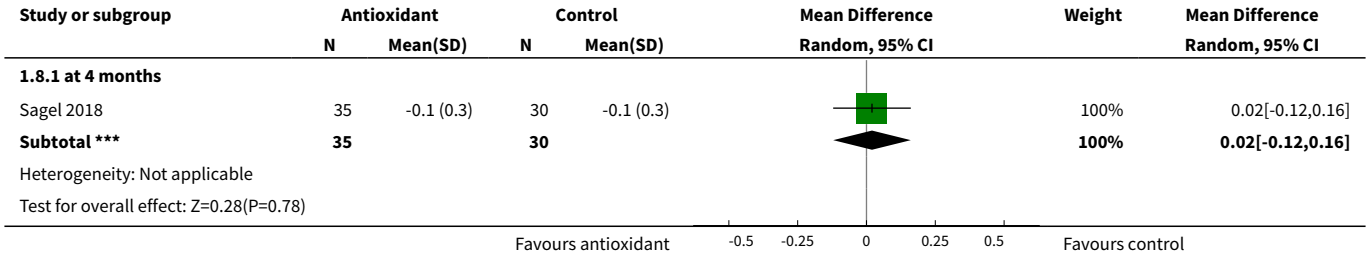
Analysis 1.6. Comparison 1 Oral antioxidants versus control, Outcome 6 Oxidative stress: lipid peroxidation (malondialdehyde) (µmol/L) mea difference to baseline).



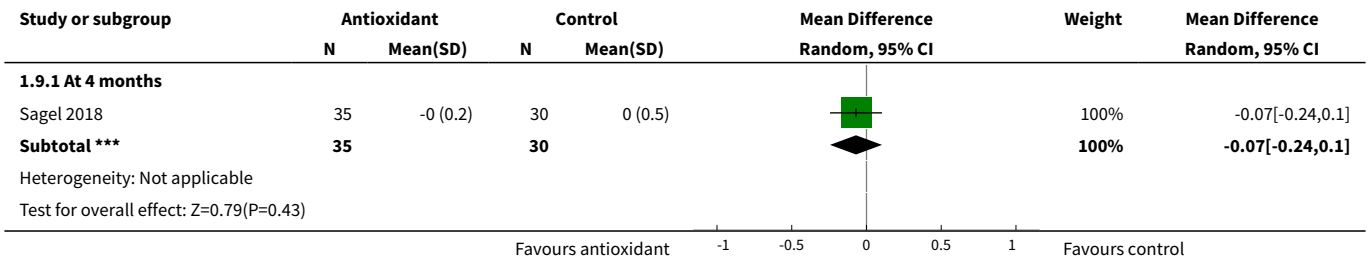
Analysis 1.7. Comparison 1 Oral antioxidants versus control, Outcome 7 Oxidative stress: urine 8-iso-PGF2α log 10 (pg/mL).



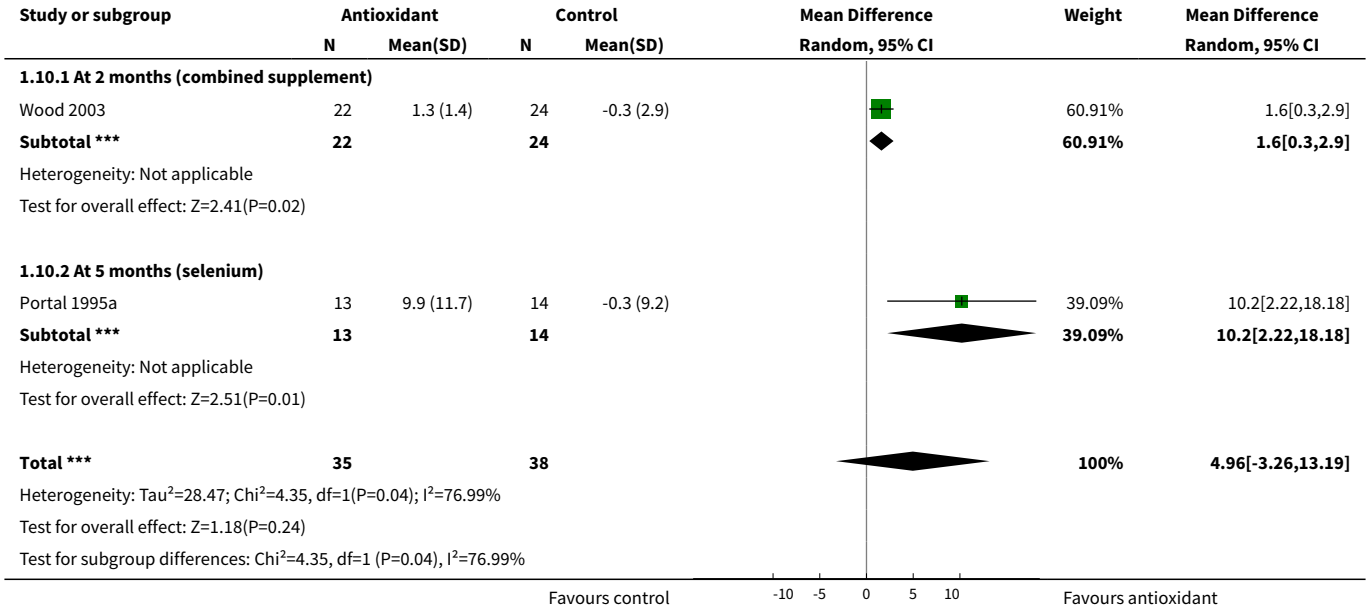
Analysis 1.8. Comparison 1 Oral antioxidants versus control, Outcome 8 Oxidative stress: sputum 8-iso-PGF2α log10 (pg/mL).



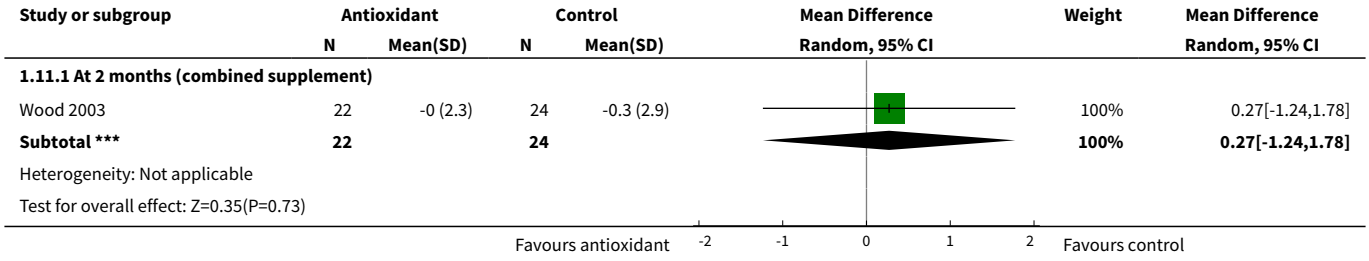
Analysis 1.9. Comparison 1 Oral antioxidants versus control, Outcome 9 Oxidative stress: sputum 8-OHdG (log10) ng/ml.



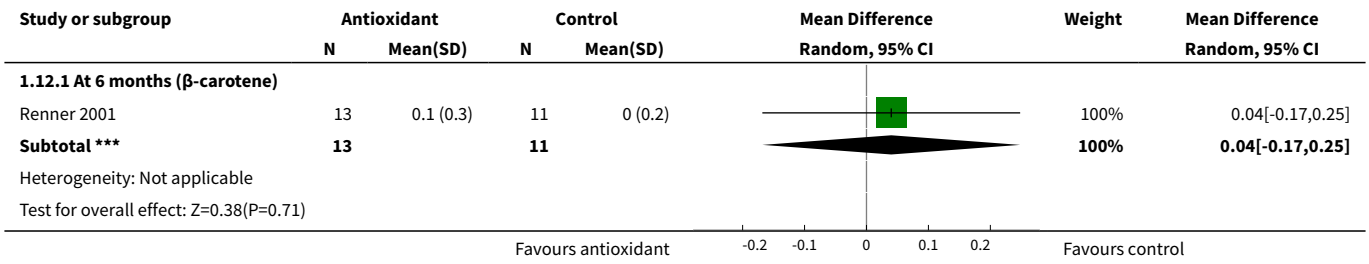
Analysis 1.10. Comparison 1 Oral antioxidants versus control, Outcome 10 Oxidative stress: enzyme function - GPX (U/g Hb).



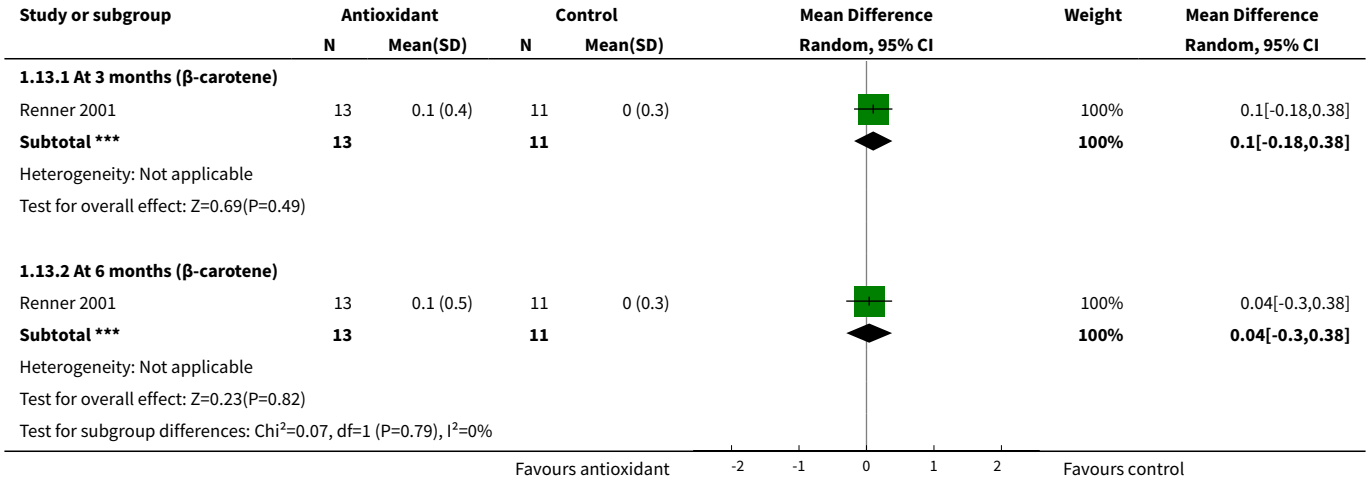
Analysis 1.11. Comparison 1 Oral antioxidants versus control, Outcome 11 Oxidative stress: enzyme function - SOD (U/mg Hb).



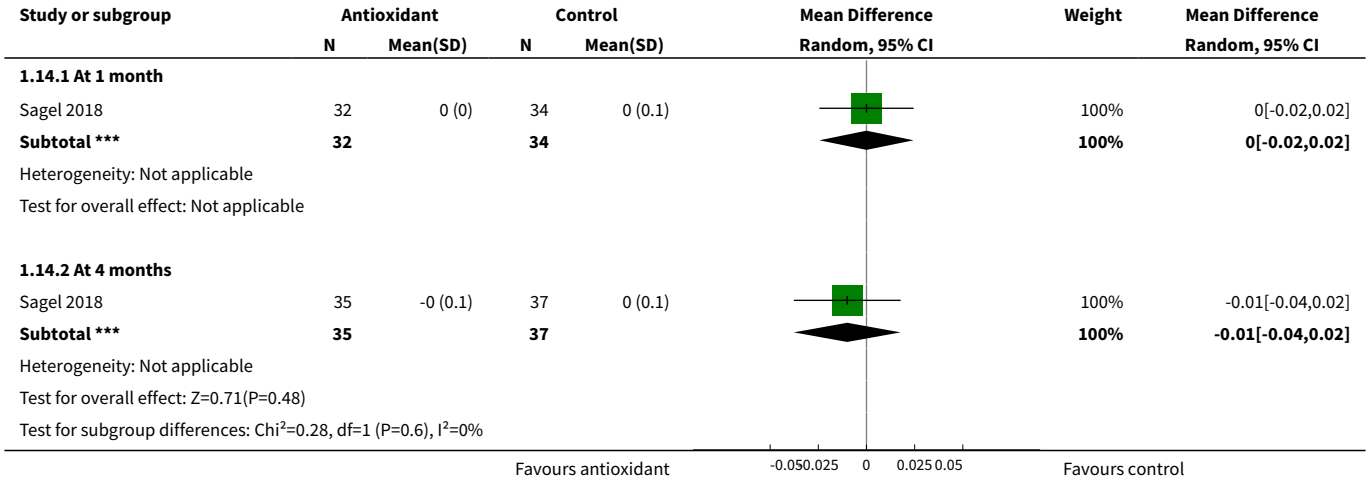
Analysis 1.12. Comparison 1 Oral antioxidants versus control, Outcome 12 Oxidative stress: potency (TEAC) (mmol/L).



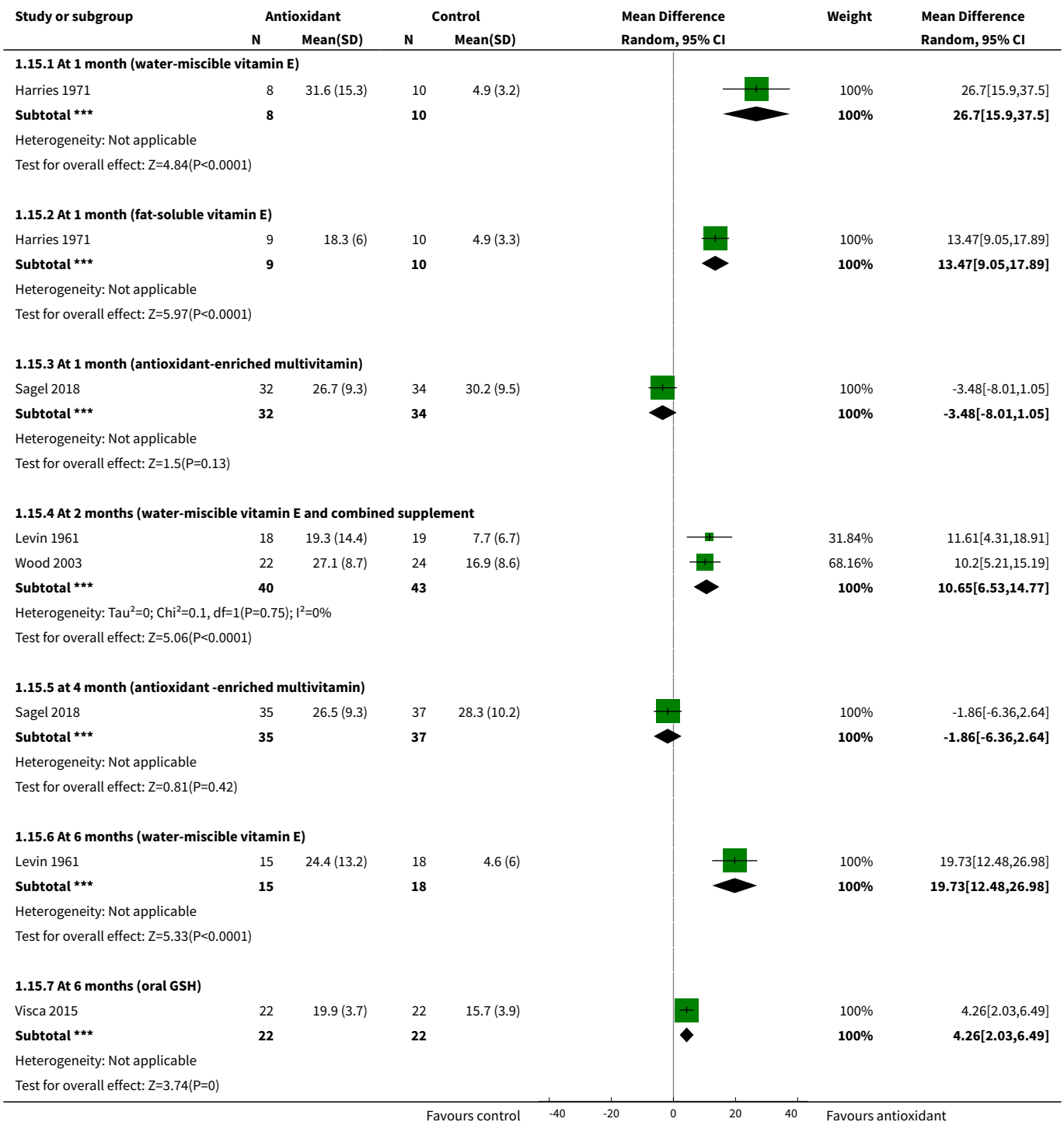
Analysis 1.13. Comparison 1 Oral antioxidants versus control, Outcome 13 Plasma total antioxidant capacity (nmol) (change from baseline).



Analysis 1.14. Comparison 1 Oral antioxidants versus control, Outcome 14 Plasma total antioxidant capacity (log10) (CRE).

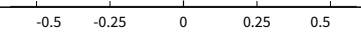


Analysis 1.15. Comparison 1 Oral antioxidants versus control, Outcome 15 Plasma antioxidant status: vitamin E (µmol/L).



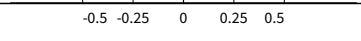
Analysis 1.16. Comparison 1 Oral antioxidants versus control, Outcome 16 Plasma antioxidant status: β-carotene (µmol/L).

Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.16.1 At 2 months (combined supplement)							
Wood 2003	22	0.1 (0.2)	24	0 (0.1)		77.11%	0.1[0.02,0.18]
Subtotal ***	**22**		**24**			**77.11%**	**0.1[0.02,0.18]**
Heterogeneity: Not applicable							
Test for overall effect: Z=2.35(P=0.02)							
1.16.2 At 6 months (β-carotene)							
Renner 2001	13	0.2 (0.1)	11	-0 (0.4)		22.89%	0.24[0.02,0.46]
Subtotal ***	**13**		**11**			**22.89%**	**0.24[0.02,0.46]**
Heterogeneity: Not applicable							
Test for overall effect: Z=2.15(P=0.03)							
Total ***	**35**		**35**			**100%**	**0.13[0.02,0.25]**
Heterogeneity: Tau^2=0; Chi^2=1.38, df=1(P=0.24); I^2=27.31%							
Test for overall effect: Z=2.24(P=0.02)							
Test for subgroup differences: Chi^2=1.38, df=1 (P=0.24), I^2=27.31%							

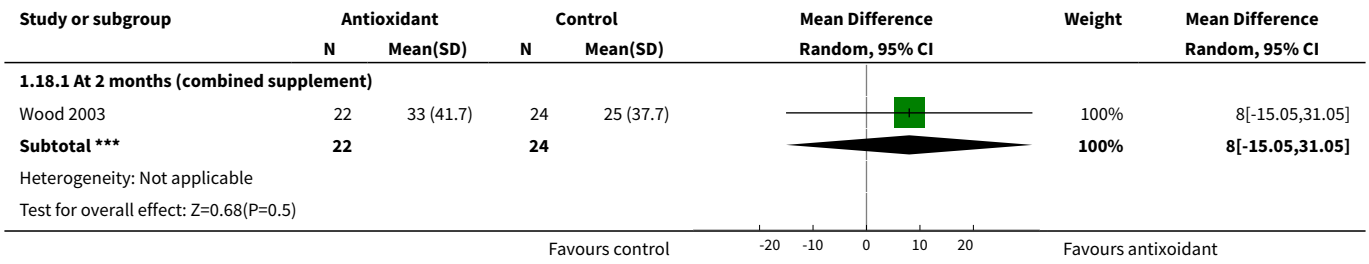


Analysis 1.17. Comparison 1 Oral antioxidants versus control, Outcome 17 Plasma antioxidant status: selenium (µmol/L).

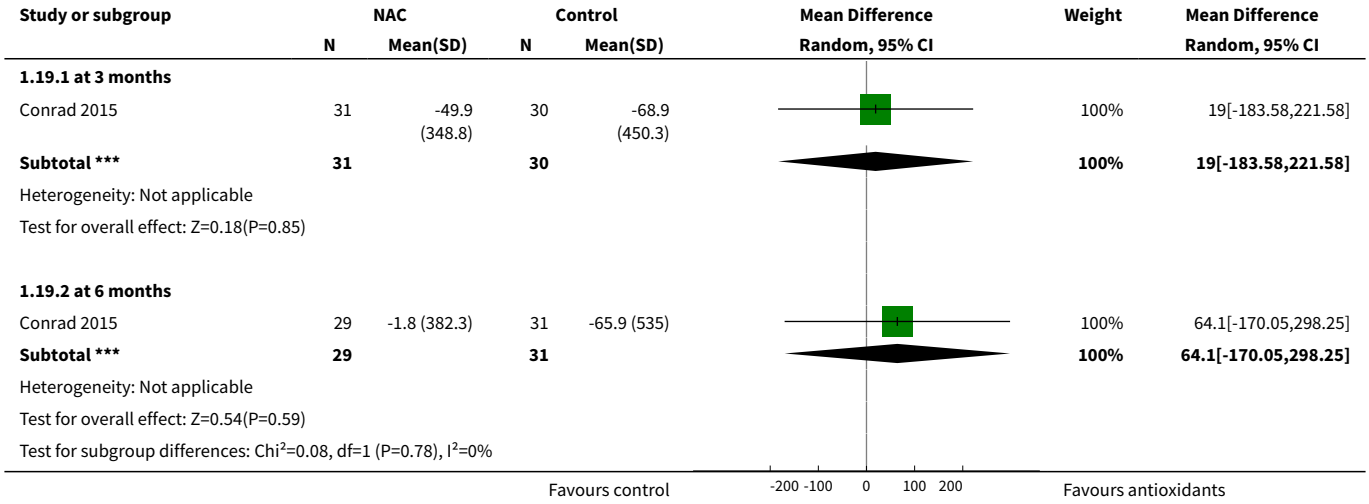
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.17.1 At 2 months (combined supplement)							
Wood 2003	22	0.5 (0.5)	24	-0.1 (0.2)		40.87%	0.6[0.39,0.81]
Subtotal ***	**22**		**24**			**40.87%**	**0.6[0.39,0.81]**
Heterogeneity: Not applicable							
Test for overall effect: Z=5.55(P<0.0001)							
1.17.2 At 5 months (selenium)							
Portal 1995a	13	0.3 (0.2)	14	-0.1 (0.1)		59.13%	0.39[0.27,0.51]
Subtotal ***	**13**		**14**			**59.13%**	**0.39[0.27,0.51]**
Heterogeneity: Not applicable							
Test for overall effect: Z=6.45(P<0.0001)							
Total ***	**35**		**38**			**100%**	**0.48[0.27,0.68]**
Heterogeneity: Tau^2=0.01; Chi^2=2.87, df=1(P=0.09); I^2=65.16%							
Test for overall effect: Z=4.61(P<0.0001)							
Test for subgroup differences: Chi^2=2.87, df=1 (P=0.09), I^2=65.16%							



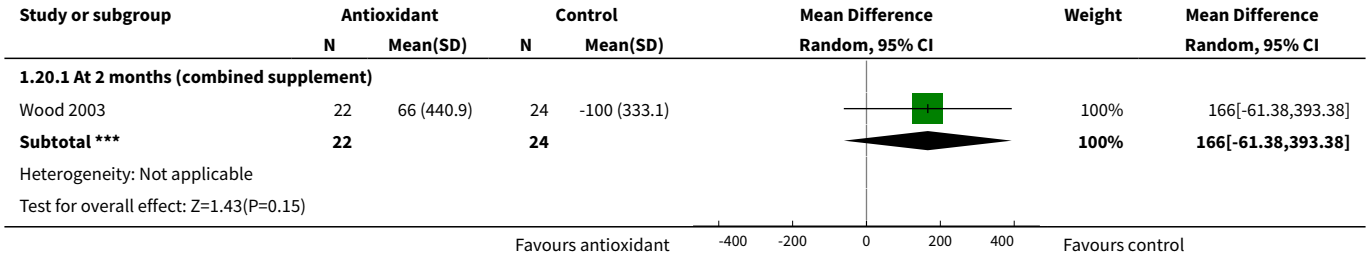
Analysis 1.18. Comparison 1 Oral antioxidants versus control, Outcome 18 Plasma antioxidant status: vitamin C (µmol/L).



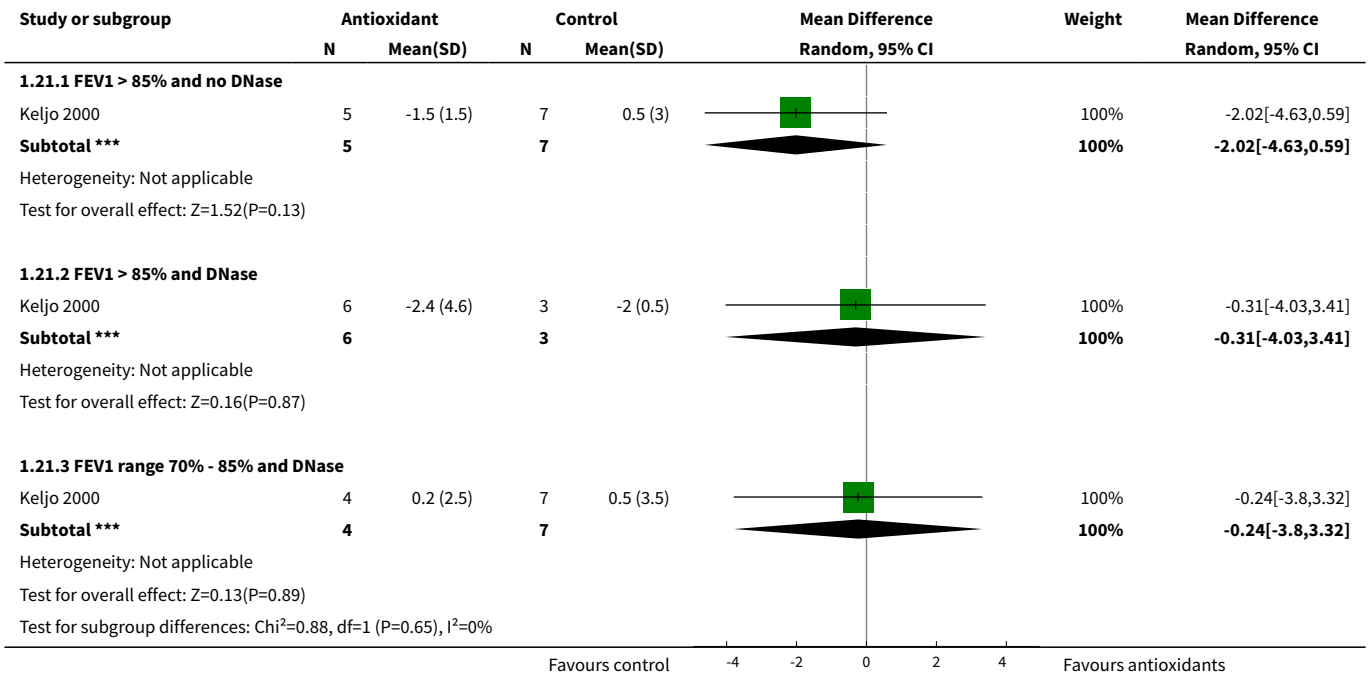
Analysis 1.19. Comparison 1 Oral antioxidants versus control, Outcome 19 Plasma antioxidant status: whole blood GSH (µmol/L) (change from baseline).



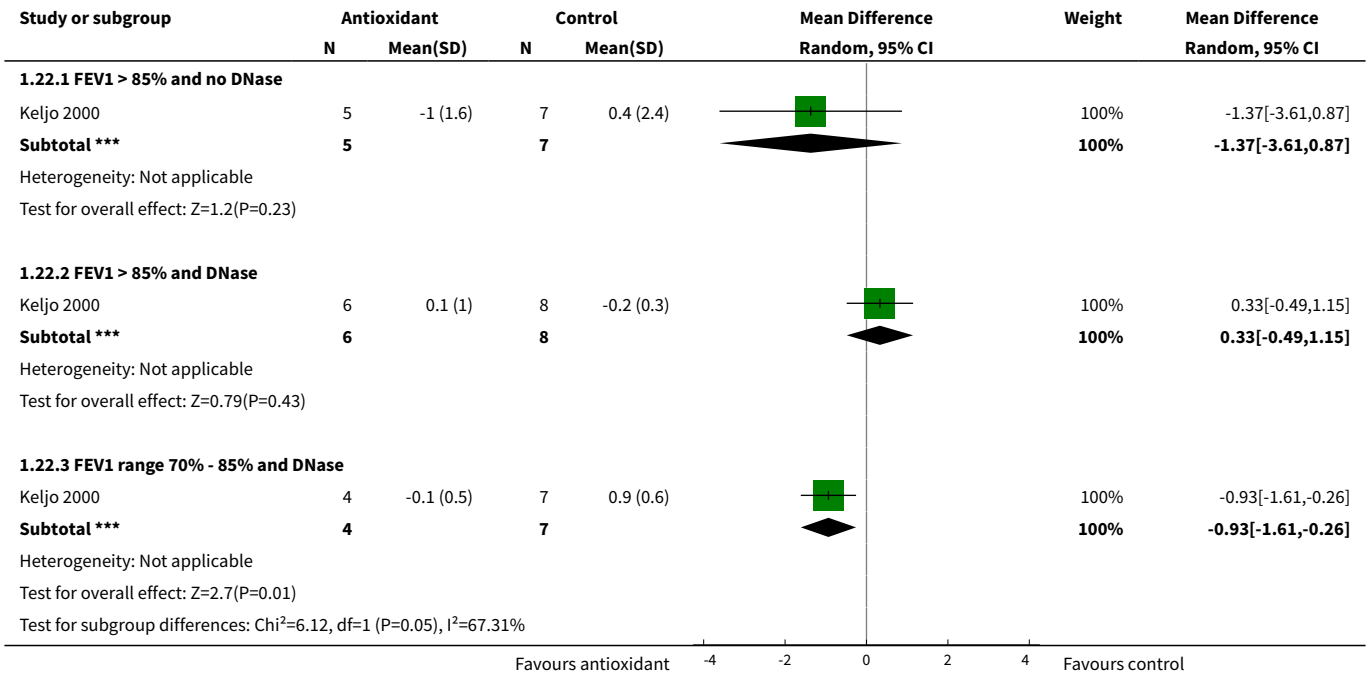
Analysis 1.20. Comparison 1 Oral antioxidants versus control, Outcome 20 Plasma antioxidant status: plasma fatty acid status (mg/L).



Analysis 1.21. Comparison 1 Oral antioxidants versus control, Outcome 21 Plasma inflammation: IL-6 (pg/mL) at 3 months (vitamin E).

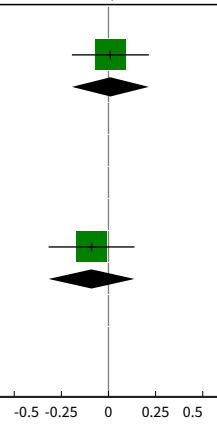


Analysis 1.22. Comparison 1 Oral antioxidants versus control, Outcome 22 Plasma inflammation: TNF-α (pg/mL) at 3 months (vitamin E).



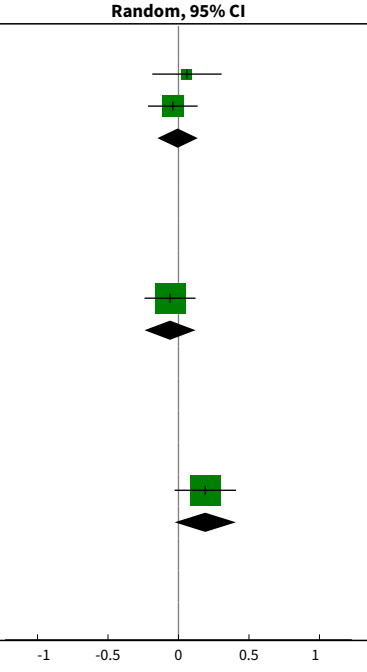
Analysis 1.23. Comparison 1 Oral antioxidants versus control, Outcome 23 Plasma IL-8 pg/mL (log 10) (change from baseline).

Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.23.1 At 3 months							
Conrad 2015	30	0.1 (0.3)	31	0.1 (0.5)		100%	0.01[-0.19,0.21]
Subtotal ***	**30**		**31**			**100%**	**0.01[-0.19,0.21]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.1(P=0.92)							
1.23.2 At 6 months							
Conrad 2015	26	-0.1 (0.4)	30	0 (0.5)		100%	-0.09[-0.32,0.14]
Subtotal ***	**26**		**30**			**100%**	**-0.09[-0.32,0.14]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.77(P=0.44)							
Test for subgroup differences: Chi^2=0.41, df=1 (P=0.52), I^2=0%							



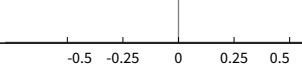
Analysis 1.24. Comparison 1 Oral antioxidants versus control, Outcome 24 Sputum IL-8 pg/ml (log 10) (per volume).

Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.24.1 At 3 months (NAC)							
Conrad 2015	30	-0 (0.6)	31	-0.1 (0.3)		33.92%	0.06[-0.19,0.31]
Dauletbaev 2009	21	0 (0.3)	10	0 (0.2)		66.08%	-0.04[-0.22,0.14]
Subtotal ***	**51**		**41**			**100%**	**-0.01[-0.15,0.14]**
Heterogeneity: Tau^2=0; Chi^2=0.42, df=1(P=0.52); I^2=0%							
Test for overall effect: Z=0.08(P=0.93)							
1.24.2 At 4 months (antioxidant enriched vitamins)							
Sagel 2018	35	0 (0.3)	30	0.1 (0.4)		100%	-0.06[-0.24,0.12]
Subtotal ***	**35**		**30**			**100%**	**-0.06[-0.24,0.12]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.65(P=0.52)							
1.24.3 At 6 months (NAC)							
Conrad 2015	26	0 (0.4)	30	-0.2 (0.4)		100%	0.19[-0.03,0.41]
Subtotal ***	**26**		**30**			**100%**	**0.19[-0.03,0.41]**
Heterogeneity: Not applicable							
Test for overall effect: Z=1.71(P=0.09)							
Test for subgroup differences: Chi^2=3.22, df=1 (P=0.2), I^2=37.96%							



Analysis 1.25. Comparison 1 Oral antioxidants versus control, Outcome 25 Sputum human neutrophil elastase (log 10) (mg/mg) per weight (change from baseline).

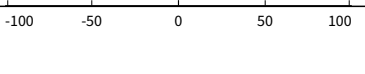
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.25.1 At 3 months							
Conrad 2015	30	-0.1 (0.4)	31	-0 (0.4)		100%	-0.04[-0.24,0.16]
Subtotal ***	**30**		**31**			**100%**	**-0.04[-0.24,0.16]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.38(P=0.7)							
1.25.2 At 6 months							
Conrad 2015	26	-0.1 (0.4)	30	-0.2 (0.5)		100%	0.11[-0.11,0.33]
Subtotal ***	**26**		**30**			**100%**	**0.11[-0.11,0.33]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.97(P=0.33)							
Test for subgroup differences: Chi^2=0.95, df=1 (P=0.33), I^2=0%							



Favours antioxidant — Favours control

Analysis 1.26. Comparison 1 Oral antioxidants versus control, Outcome 26 Sputum neutrophil count (logarithm).

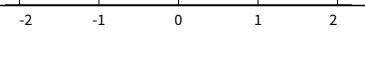
Study or subgroup	Experimental N	Experimental Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.26.1 at 3 months							
Conrad 2015	31	-4 (23.9)	32	-5.9 (15.5)		100%	1.9[-8.08,11.88]
Subtotal ***	**31**		**32**			**100%**	**1.9[-8.08,11.88]**
Heterogeneity: Tau^2=0; Chi^2=0, df=0(P<0.0001); I^2=100%							
Test for overall effect: Z=0.37(P=0.71)							
1.26.2 at 6 months							
Conrad 2015	29	-2 (29)	32	-4.6 (28.5)		100%	2.6[-11.85,17.05]
Subtotal ***	**29**		**32**			**100%**	**2.6[-11.85,17.05]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.35(P=0.72)							
Test for subgroup differences: Chi^2=0.01, df=1 (P=0.94), I^2=0%							



Favours control — Favours antioxidant

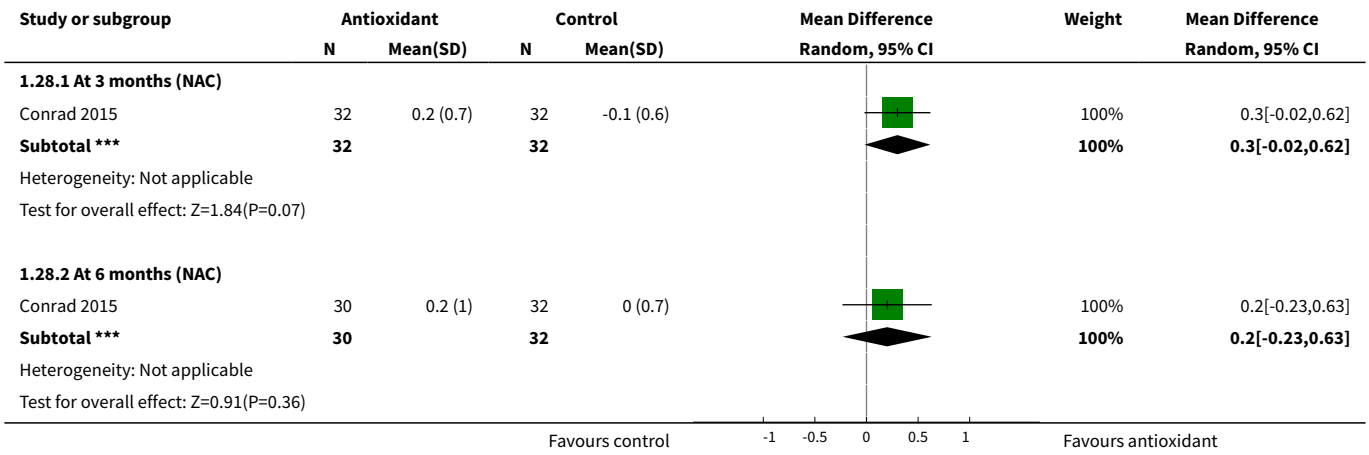
Analysis 1.27. Comparison 1 Oral antioxidants versus control, Outcome 27 Sputum myeloperoxidase (MPO) levels (log 10) (ng/mL).

Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
1.27.1 At 4 months (multivitamins with antioxidants versus multivitamin alone							
Sagel 2018	35	-0.1 (0.7)	30	0 (0.8)		100%	-0.13[-0.48,0.22]
Subtotal ***	**35**		**30**			**100%**	**-0.13[-0.48,0.22]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.73(P=0.47)							

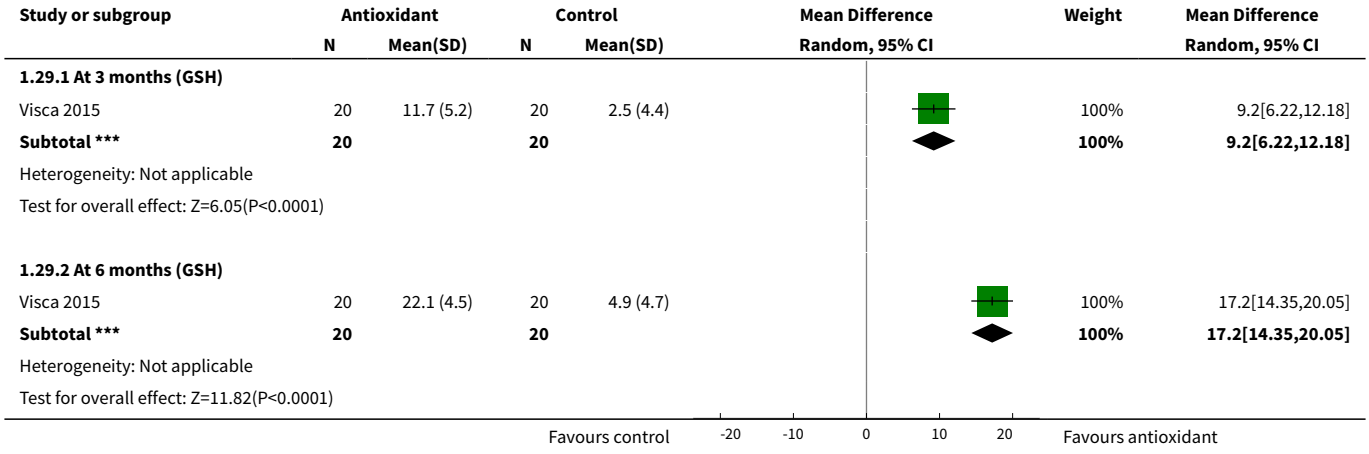


Favours antioxidant — Favours control

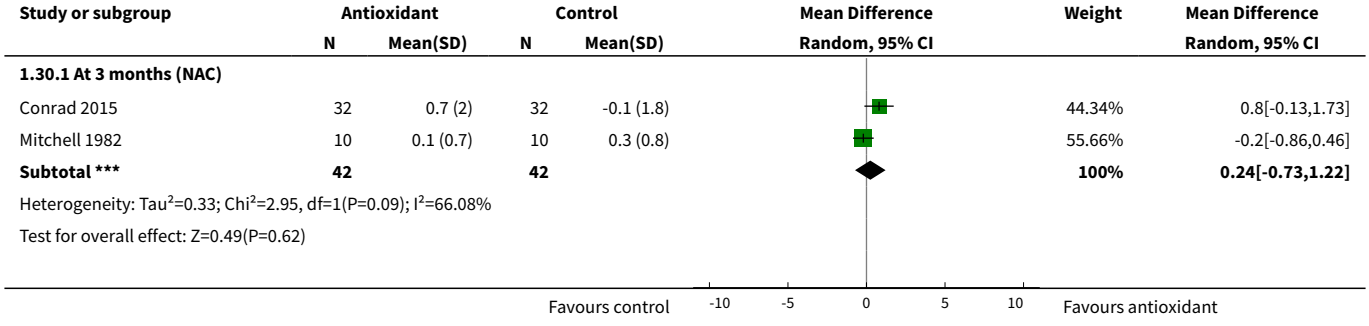
Analysis 1.28. Comparison 1 Oral antioxidants versus control, Outcome 28 Nutritional status: BMI (change from baseline).

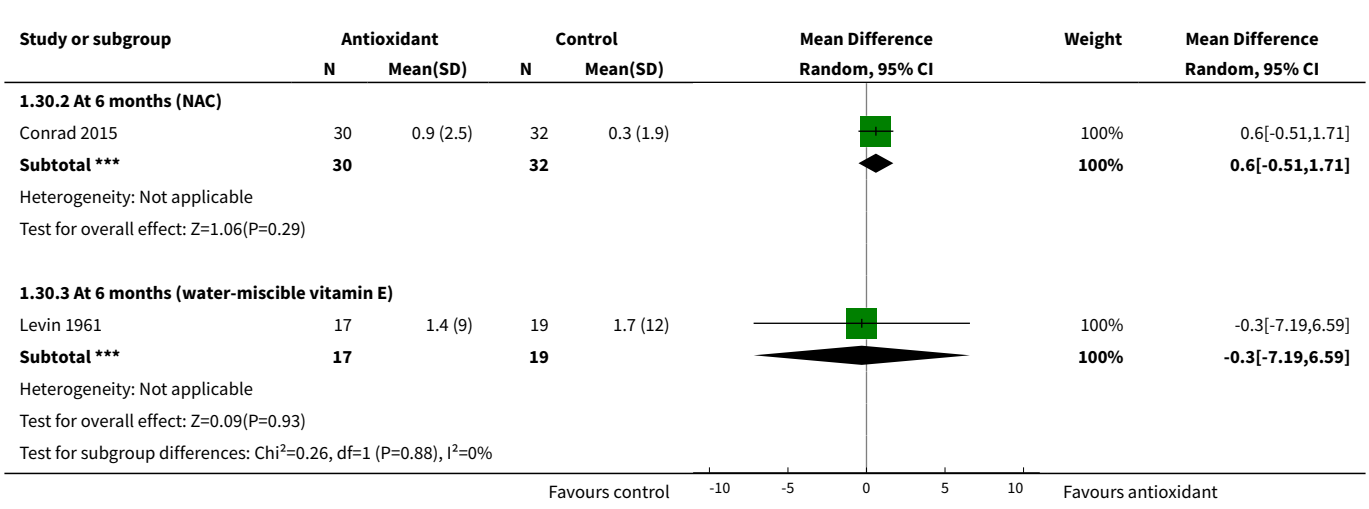


Analysis 1.29. Comparison 1 Oral antioxidants versus control, Outcome 29 Nutritional status: BMI percentile (change from baseline).

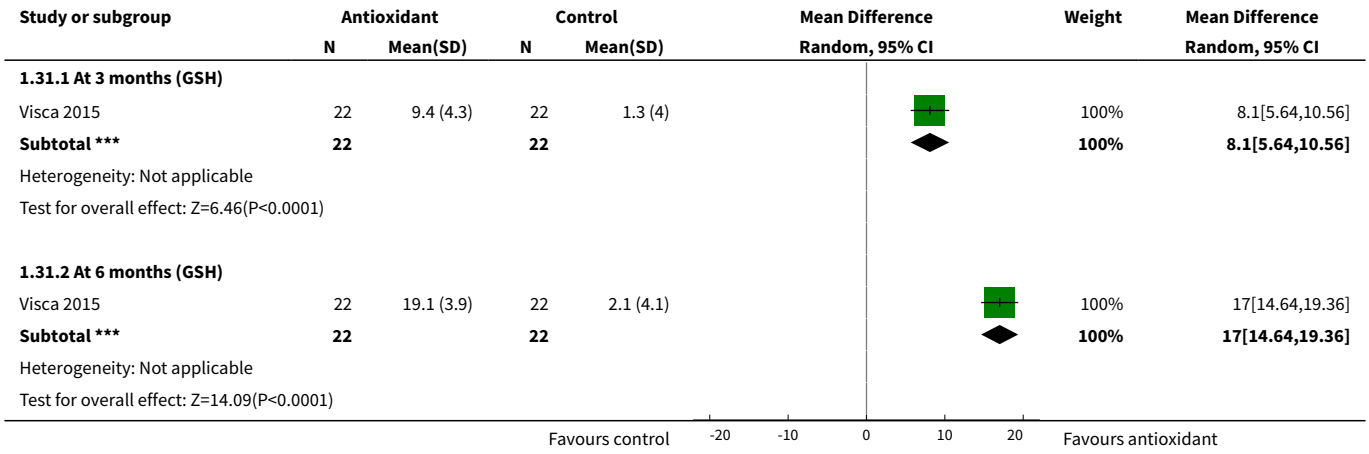


Analysis 1.30. Comparison 1 Oral antioxidants versus control, Outcome 30 Nutritional status: weight (kg) (change from baseline).

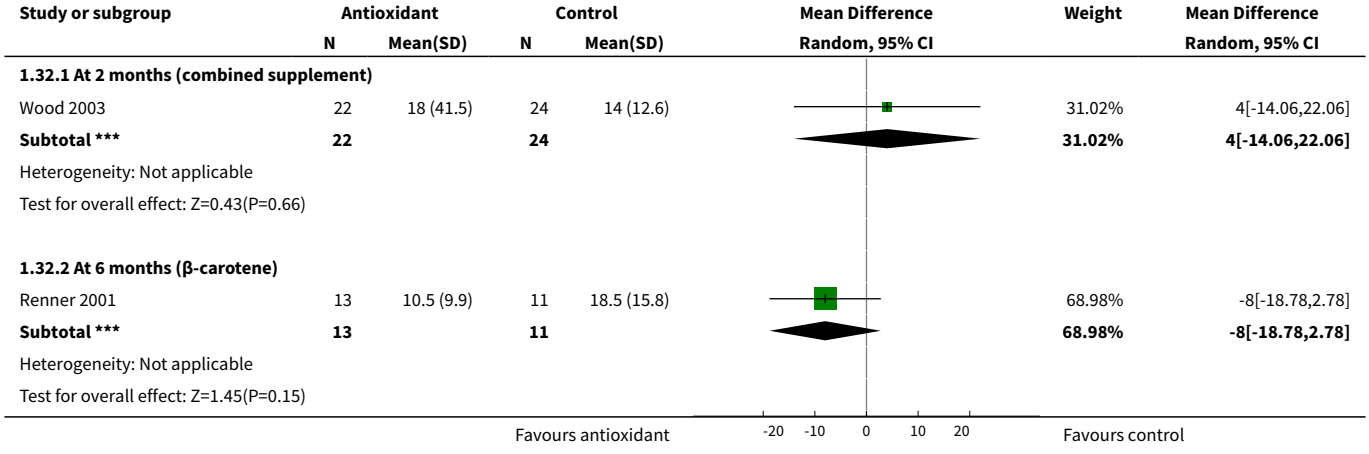


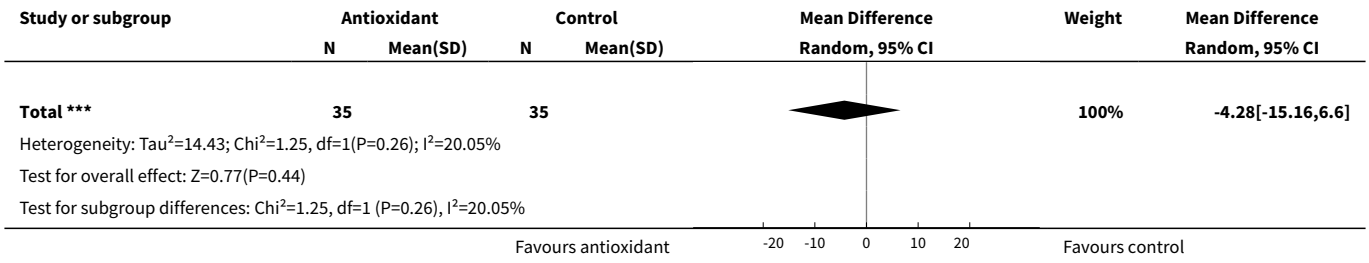


Analysis 1.31. Comparison 1 Oral antioxidants versus control, Outcome 31 Nutritional status: weight percentile (change from baseline).

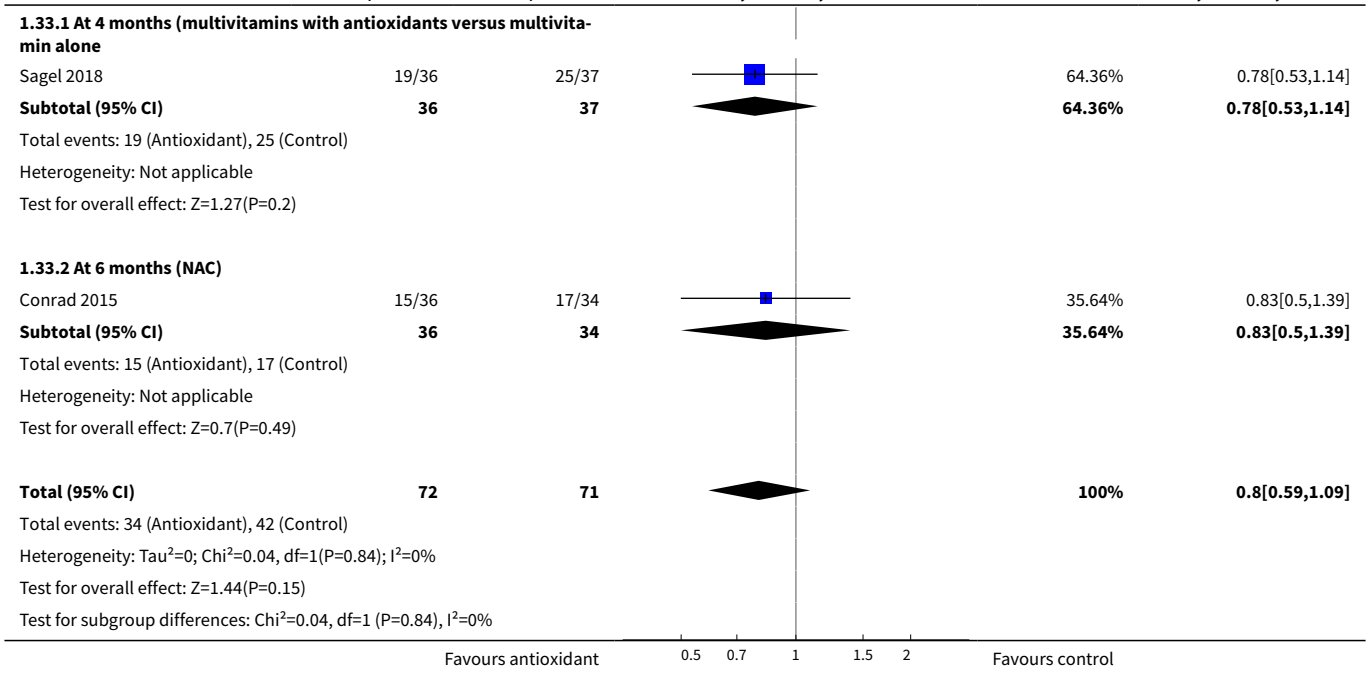


Analysis 1.32. Comparison 1 Oral antioxidants versus control, Outcome 32 Antibiotic days per participant.

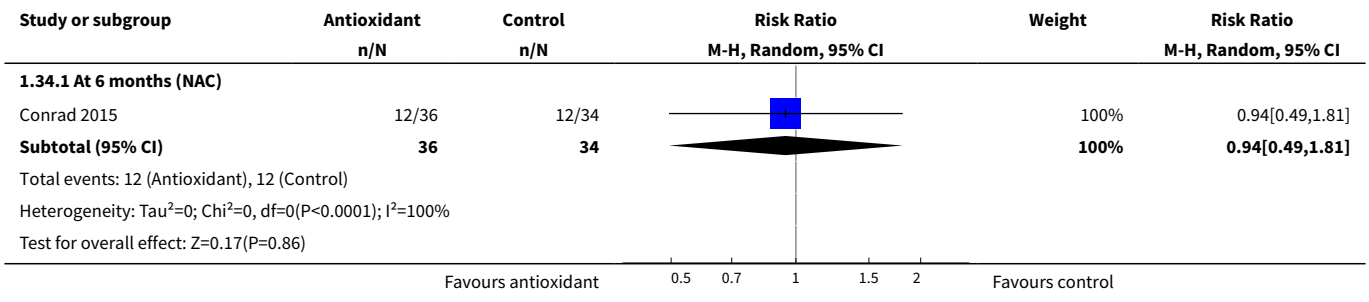




Analysis 1.33. Comparison 1 Oral antioxidants versus control, Outcome 33 Number of participants with at least one exacerbation.

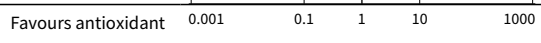


Analysis 1.34. Comparison 1 Oral antioxidants versus control, Outcome 34 Number of hospitalizations.



Analysis 1.35. Comparison 1 Oral antioxidants versus control, Outcome 35 Adverse effects.

Study or subgroup	Antioxidant n/N	Control n/N	Odds Ratio M-H, Random, 95% CI	Weight	Odds Ratio M-H, Random, 95% CI
1.35.1 Sinusitis					
Conrad 2015	1/36	0/34		19.28%	2.92[0.11,74.05]
Keljo 2000	2/19	2/19		46.99%	1[0.13,7.94]
Sagel 2018	2/36	1/37		33.73%	2.12[0.18,24.44]
Subtotal (95% CI)	**91**	**90**		**100%**	**1.58[0.38,6.55]**
Total events: 5 (Antioxidant), 3 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0.38, df=2(P=0.83); I^2=0%					
Test for overall effect: Z=0.63(P=0.53)					
1.35.2 Distal intestinal obstruction syndrome					
Conrad 2015	0/36	1/34		24.61%	0.31[0.01,7.77]
Keljo 2000	1/19	0/19		24.18%	3.16[0.12,82.64]
Sagel 2018	1/36	4/37		51.21%	0.24[0.03,2.22]
Subtotal (95% CI)	**91**	**90**		**100%**	**0.47[0.09,2.34]**
Total events: 2 (Antioxidant), 5 (Control)					
Heterogeneity: Tau^2=0; Chi^2=1.74, df=2(P=0.42); I^2=0%					
Test for overall effect: Z=0.92(P=0.36)					
1.35.3 Diahorrea					
Conrad 2015	4/36	3/34		49.21%	1.29[0.27,6.25]
Keljo 2000	1/19	0/19		11.48%	3.16[0.12,82.64]
Sagel 2018	4/36	2/37		39.31%	2.19[0.37,12.76]
Subtotal (95% CI)	**91**	**90**		**100%**	**1.76[0.58,5.32]**
Total events: 9 (Antioxidant), 5 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0.33, df=2(P=0.85); I^2=0%					
Test for overall effect: Z=1(P=0.32)					
1.35.4 Pulmonary exacerbations					
Keljo 2000	1/19	1/19		10.01%	1[0.06,17.25]
Sagel 2018	19/36	25/37		89.99%	0.54[0.21,1.39]
Subtotal (95% CI)	**55**	**56**		**100%**	**0.57[0.23,1.41]**
Total events: 20 (Antioxidant), 26 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0.17, df=1(P=0.68); I^2=0%					
Test for overall effect: Z=1.22(P=0.22)					
1.35.5 Elevated liver enzymes (ALT)					
Conrad 2015	1/36	0/34		50.44%	2.92[0.11,74.05]
Keljo 2000	1/19	0/19		49.56%	3.16[0.12,82.64]
Subtotal (95% CI)	**55**	**53**		**100%**	**3.04[0.31,30.19]**
Total events: 2 (Antioxidant), 0 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0, df=1(P=0.97); I^2=0%					
Test for overall effect: Z=0.95(P=0.34)					
Test for subgroup differences: Chi^2=4.63, df=1 (P=0.33), I^2=13.59%					



Comparison 2. Inhaled antioxidant (glutathione) versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Lung function: FEV_1 (L) (change from baseline)	3		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.1 At 1 month	3	258	Mean Difference (IV, Random, 95% CI)	0.05 [-0.01, 0.11]
1.2 At 3 months	3	258	Mean Difference (IV, Random, 95% CI)	0.09 [0.03, 0.15]
1.3 At 6 months	3	258	Mean Difference (IV, Random, 95% CI)	0.07 [0.00, 0.14]
1.4 At 9 months	2	105	Mean Difference (IV, Random, 95% CI)	0.03 [-0.14, 0.20]
1.5 At 12 months	2	105	Mean Difference (IV, Random, 95% CI)	-0.00 [-0.13, 0.12]
2 Lung function: FEV_1 (% predicted) (change from baseline)	4		Mean Difference (IV, Random, 95% CI)	Subtotals only
2.1 At 1 month	3	258	Mean Difference (IV, Random, 95% CI)	1.91 [-0.07, 3.88]
2.2 At 2 months	1	16	Mean Difference (IV, Random, 95% CI)	0.90 [-6.45, 8.25]
2.3 At 3 months	3	258	Mean Difference (IV, Random, 95% CI)	3.50 [1.38, 5.62]
2.4 At 6 months	3	258	Mean Difference (IV, Random, 95% CI)	2.30 [-0.12, 4.71]
2.5 At 9 months	2	105	Mean Difference (IV, Random, 95% CI)	2.52 [-4.61, 9.65]
2.6 At 12 months	2	105	Mean Difference (IV, Random, 95% CI)	2.96 [-2.54, 8.46]
3 Lung function FVC (L) (change from baseline)	3		Mean Difference (IV, Random, 95% CI)	Subtotals only
3.1 At 1 month	3	258	Mean Difference (IV, Random, 95% CI)	0.05 [-0.01, 0.12]
3.2 At 3 months	3	258	Mean Difference (IV, Random, 95% CI)	0.08 [0.01, 0.16]
3.3 At 6 months	3	258	Mean Difference (IV, Random, 95% CI)	0.05 [-0.03, 0.13]
3.4 At 9 months	2	105	Mean Difference (IV, Random, 95% CI)	0.01 [-0.17, 0.19]
3.5 At 12 months	2	105	Mean Difference (IV, Random, 95% CI)	-0.01 [-0.10, 0.09]
4 Lung function FVC (% predicted) (change from baseline)	4		Mean Difference (IV, Random, 95% CI)	Subtotals only
4.1 At 1 month	3	258	Mean Difference (IV, Random, 95% CI)	2.12 [-0.23, 4.47]
4.2 At 2 months	1	16	Mean Difference (IV, Random, 95% CI)	0.60 [-6.53, 7.73]
4.3 At 3 months	3	258	Mean Difference (IV, Random, 95% CI)	3.60 [1.33, 5.88]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.4 At 6 months	3	258	Mean Difference (IV, Random, 95% CI)	3.33 [-0.62, 7.27]
4.5 At 9 months	2	105	Mean Difference (IV, Random, 95% CI)	5.48 [-1.76, 12.73]
4.6 At 12 months	2	105	Mean Difference (IV, Random, 95% CI)	4.27 [-0.00, 8.54]
5 QoL total score (change from baseline)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
5.1 at 1 month	1	153	Mean Difference (IV, Random, 95% CI)	2.2 [-0.23, 4.63]
5.2 at 3 months	1	153	Mean Difference (IV, Random, 95% CI)	1.20 [-1.46, 3.86]
5.3 at 6 months	1	153	Mean Difference (IV, Random, 95% CI)	0.8 [-1.63, 3.23]
6 QoL respiratory score (change from baseline)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
6.1 at 1 month	1	153	Mean Difference (IV, Random, 95% CI)	2.7 [-2.15, 7.55]
6.2 at 3 months	1	153	Mean Difference (IV, Random, 95% CI)	-0.5 [-4.80, 3.80]
6.3 at 6 months	1	153	Mean Difference (IV, Random, 95% CI)	-3.3 [-8.05, 1.45]
7 Oxidative stress markers in exhaled breath condensate: H_2O_2 (µM)	2		Mean Difference (IV, Random, 95% CI)	Subtotals only
7.1 At 12 months	2	105	Mean Difference (IV, Random, 95% CI)	-0.16 [-0.40, 0.09]
8 Sputum oxidative stress: lipid peroxidation (8-iso-prostan)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
8.1 At 3 months	1	42	Mean Difference (IV, Random, 95% CI)	-51.3 [-128.22, 25.62]
8.2 At 6 months	1	42	Mean Difference (IV, Random, 95% CI)	-5.6 [-95.70, 84.50]
9 Sputum antioxidant status: free glutathione in sputum (pM)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
9.1 At 1 month	1	55	Mean Difference (IV, Random, 95% CI)	131.3 [-36.81, 299.41]
9.2 At 3 months	1	47	Mean Difference (IV, Random, 95% CI)	81.4 [-8.01, 170.81]
9.3 At 6 months	1	50	Mean Difference (IV, Random, 95% CI)	59.1 [3.68, 114.52]
10 Sputum antioxidant status: total glutathione in sputum (pM)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only

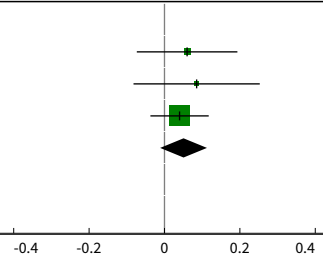
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
10.1 At 1 month	1	54	Mean Difference (IV, Random, 95% CI)	405.3 [105.27, 705.33]
10.2 At 3 months	1	48	Mean Difference (IV, Random, 95% CI)	329.2 [167.04, 491.36]
10.3 At 6 months	1	50	Mean Difference (IV, Random, 95% CI)	273.5 [-19.52, 566.52]
11 Sputum antioxidant status: glutathione in sputum neutrophils (MFI)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
11.1 At 1 month	1	14	Mean Difference (IV, Random, 95% CI)	0.8 [-0.06, 1.66]
11.2 At 3 months	1	15	Mean Difference (IV, Random, 95% CI)	3.70 [0.27, 7.13]
11.3 At 6 months	1	16	Mean Difference (IV, Random, 95% CI)	4.4 [1.52, 7.28]
12 Plasma antioxidant status: free glutathione (pM)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
12.1 At 6 months	1	57	Mean Difference (IV, Random, 95% CI)	2.20 [-1.44, 5.84]
13 Plasma antioxidant status: total glutathione (pM)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
13.1 At 6 months	1	57	Mean Difference (IV, Random, 95% CI)	0.8 [-2.07, 3.67]
14 Plasma antioxidant status: glutathione in blood neutrophils (MFI)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
14.1 At 6 months	1	13	Mean Difference (IV, Random, 95% CI)	-2.9 [-12.39, 6.59]
15 Sputum oxidative stress: protein carbonyls (U)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
15.1 At 1 month	1	49	Mean Difference (IV, Random, 95% CI)	4.20 [-7.92, 16.32]
15.2 At 3 months	1	44	Mean Difference (IV, Random, 95% CI)	-0.10 [-13.20, 13.00]
15.3 At 6 months	1	41	Mean Difference (IV, Random, 95% CI)	10.70 [-2.63, 24.03]
16 Local inflammation: cytokines in sputum IL-8 (pg/mL)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
16.1 At 6 months	1	53	Mean Difference (IV, Random, 95% CI)	-478.3 [-1536.75, 580.15]
17 Local inflammation: cytokines in sputum IL-10 (pg/mL)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only

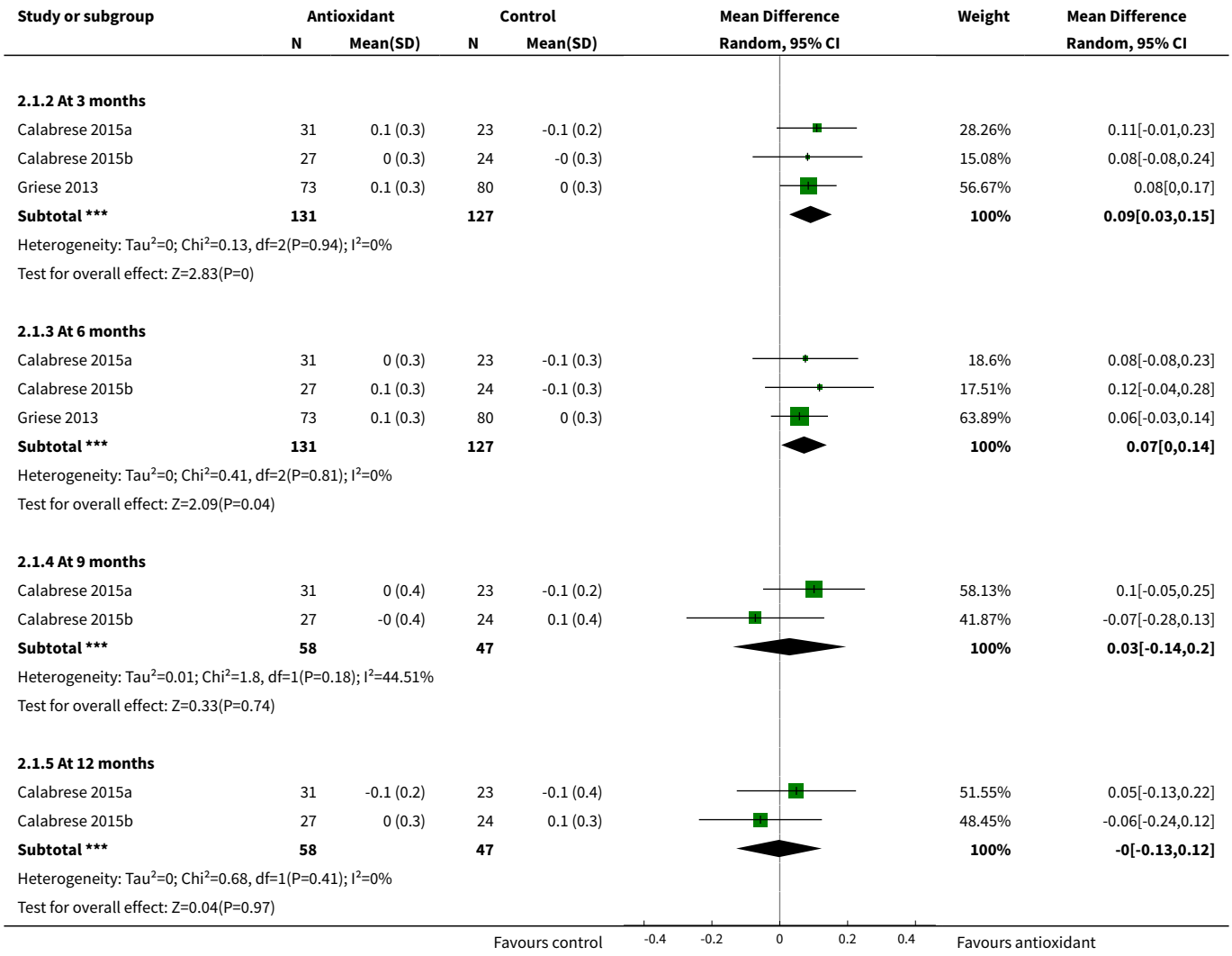
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
17.1 At 6 months	1	53	Mean Difference (IV, Random, 95% CI)	-0.20 [-10.12, 9.72]
18 Local inflammation: cytokines in sputum TNF-α (pg/mL)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
18.1 At 6 months	1	53	Mean Difference (IV, Random, 95% CI)	19.8 [-50.33, 89.93]
19 Nutritional status: BMI (change from baseline)	3		Mean Difference (IV, Random, 95% CI)	Subtotals only
19.1 At 2 months	1	16	Mean Difference (IV, Random, 95% CI)	0.10 [-0.74, 0.94]
19.2 At 12 months	2	105	Mean Difference (IV, Random, 95% CI)	0.04 [-8.20, 8.27]
20 Nutritional status: weight (kg)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
20.1 At 1 month	1	153	Mean Difference (IV, Random, 95% CI)	0.1 [-0.23, 0.43]
20.2 At 3 months	1	153	Mean Difference (IV, Random, 95% CI)	1.0 [0.39, 1.61]
20.3 At 6 months	1	153	Mean Difference (IV, Random, 95% CI)	0.30 [-0.37, 0.97]
21 Number of pulmonary exacerbations during the study	3		Mean Difference (IV, Random, 95% CI)	Subtotals only
21.1 At 6 months	1	153	Mean Difference (IV, Random, 95% CI)	-0.09 [-0.30, 0.12]
21.2 At 12 months	2	105	Mean Difference (IV, Random, 95% CI)	-0.18 [-0.60, 0.23]
22 Time to first pulmonary exacerbation (days)	2		Mean Difference (IV, Random, 95% CI)	Subtotals only
22.1 At 12 months	2	105	Mean Difference (IV, Random, 95% CI)	-6.74 [-48.76, 35.27]
23 Adverse events	4		Peto Odds Ratio (Peto, Fixed, 95% CI)	Subtotals only
23.1 Hospitalization for non-acute pulmonary exacerbations	1	19	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.42 [0.04, 4.63]
23.2 Rhinitis/sinusitis or upper respiratory tract infection	3	223	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.04 [0.43, 2.55]
23.3 Cough	2	172	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.14 [0.63, 2.08]
23.4 Pharyngitis	2	172	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.12 [0.61, 2.06]
23.5 Stomach pain/cramps	2	70	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.71 [0.19, 2.56]
23.6 Headache	3	223	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.08 [0.55, 2.13]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
23.7 Chest tightness/bronchospasm	2	70	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.41 [0.08, 2.28]
23.8 Nose bleed	2	70	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.31 [0.06, 1.59]
23.9 Shortness of breath	1	19	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.42 [0.04, 4.63]
23.10 Sputum increase	1	153	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.13 [0.55, 2.32]
23.11 Pyrexia	1	153	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.76 [0.69, 4.49]
23.12 Haemoptysis	3	258	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.94 [0.49, 1.79]
23.13 Lung disorder	1	153	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.98 [0.38, 2.57]
23.14 Sputum abnormal	1	153	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.59 [0.61, 4.14]
23.15 Infection	1	153	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.42 [0.53, 3.80]
23.16 Rales	1	153	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.11 [0.39, 3.11]
23.17 Oropharyngeal pain	1	153	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.48 [0.17, 1.35]
23.18 Condition aggravated	1	153	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.06 [0.52, 2.16]
23.19 Pancreatitis	1	51	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.12 [0.00, 6.06]
23.20 Constipation	1	51	Peto Odds Ratio (Peto, Fixed, 95% CI)	6.61 [0.13, 335.50]
23.21 Pityriasis	1	51	Peto Odds Ratio (Peto, Fixed, 95% CI)	6.61 [0.13, 335.50]
23.22 Impaired glucose tolerance	1	51	Peto Odds Ratio (Peto, Fixed, 95% CI)	6.61 [0.13, 335.50]
23.23 Distal intestinal obstruction	2	105	Peto Odds Ratio (Peto, Fixed, 95% CI)	6.14 [0.62, 60.94]

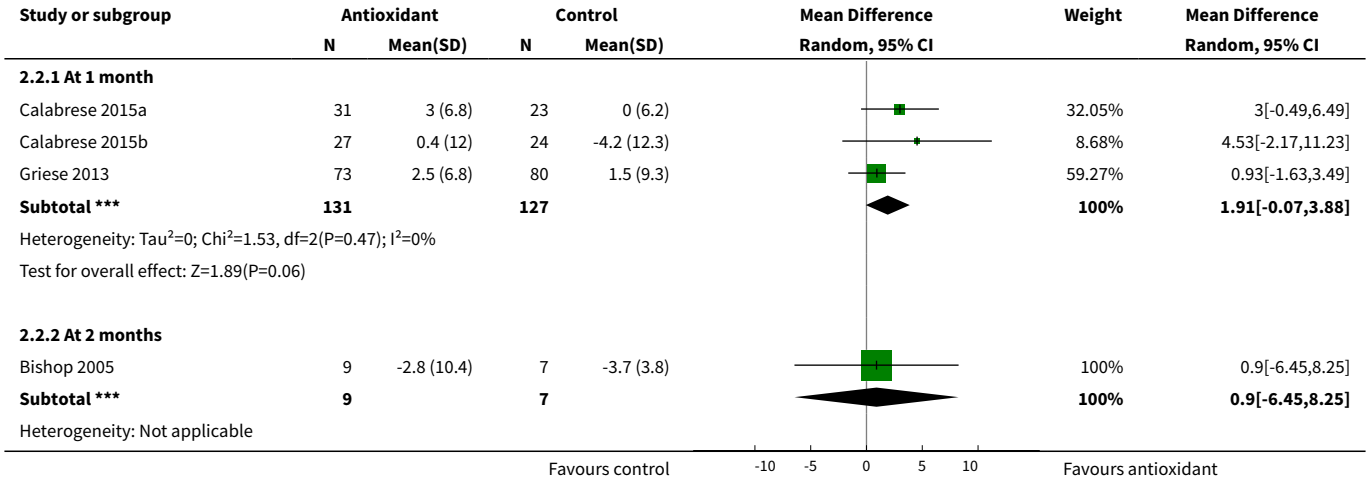
Analysis 2.1. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 1 Lung function: FEV_1 (L) (change from baseline).

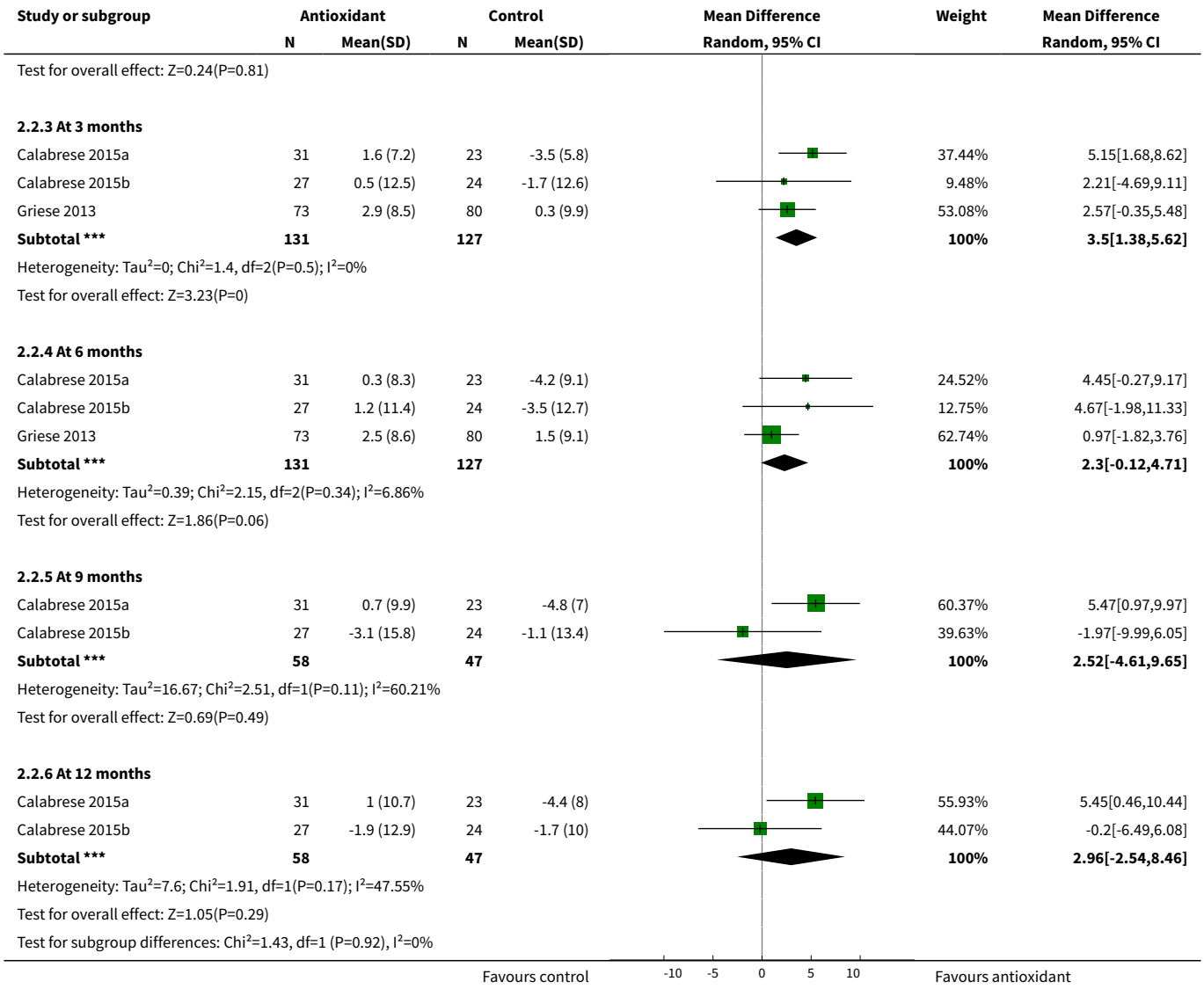
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
2.1.1 At 1 month							
Calabrese 2015a	31	0.1 (0.3)	23	0 (0.2)		21.68%	0.06[-0.07,0.19]
Calabrese 2015b	27	0 (0.3)	24	-0.1 (0.3)		13.76%	0.09[-0.08,0.25]
Griese 2013	73	0.1 (0.2)	80	0 (0.3)		64.55%	0.04[-0.04,0.12]
Subtotal ***	**131**		**127**			**100%**	**0.05[-0.01,0.11]**
Heterogeneity: Tau^2=0; Chi^2=0.26, df=2(P=0.88); I^2=0%							
Test for overall effect: Z=1.6(P=0.11)							



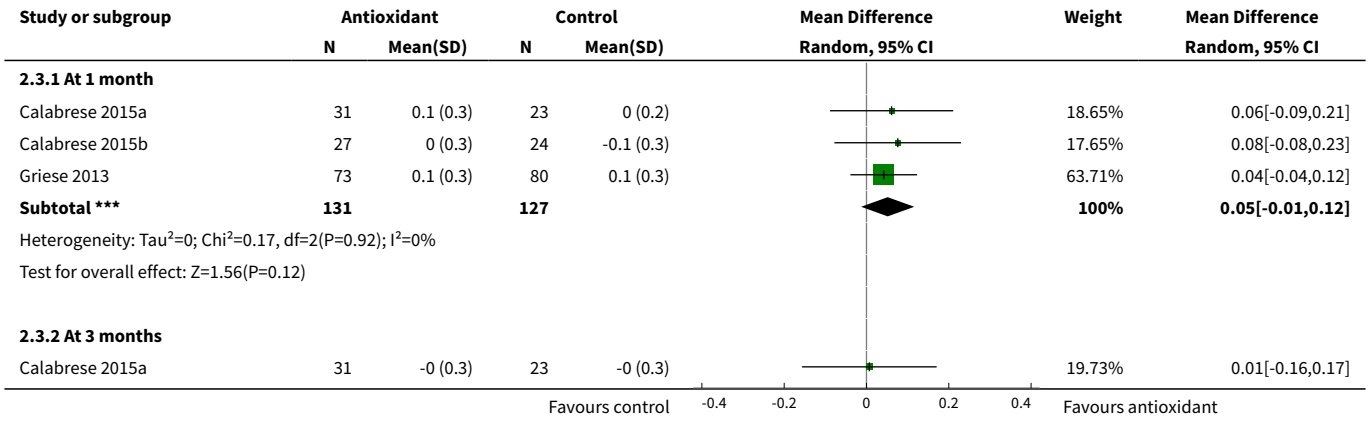


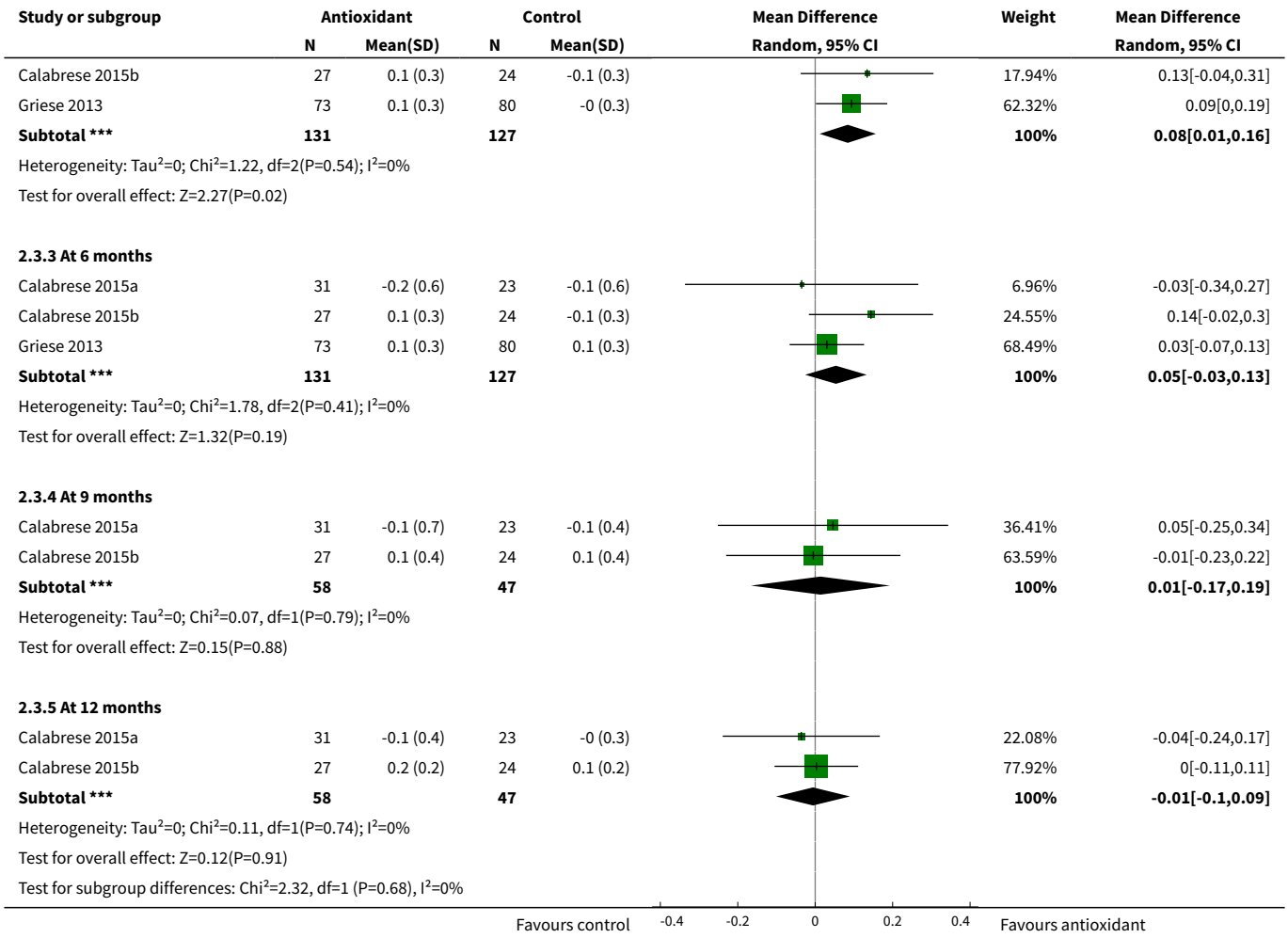
Analysis 2.2. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 2 Lung function: FEV_1 (% predicted) (change from baseline).



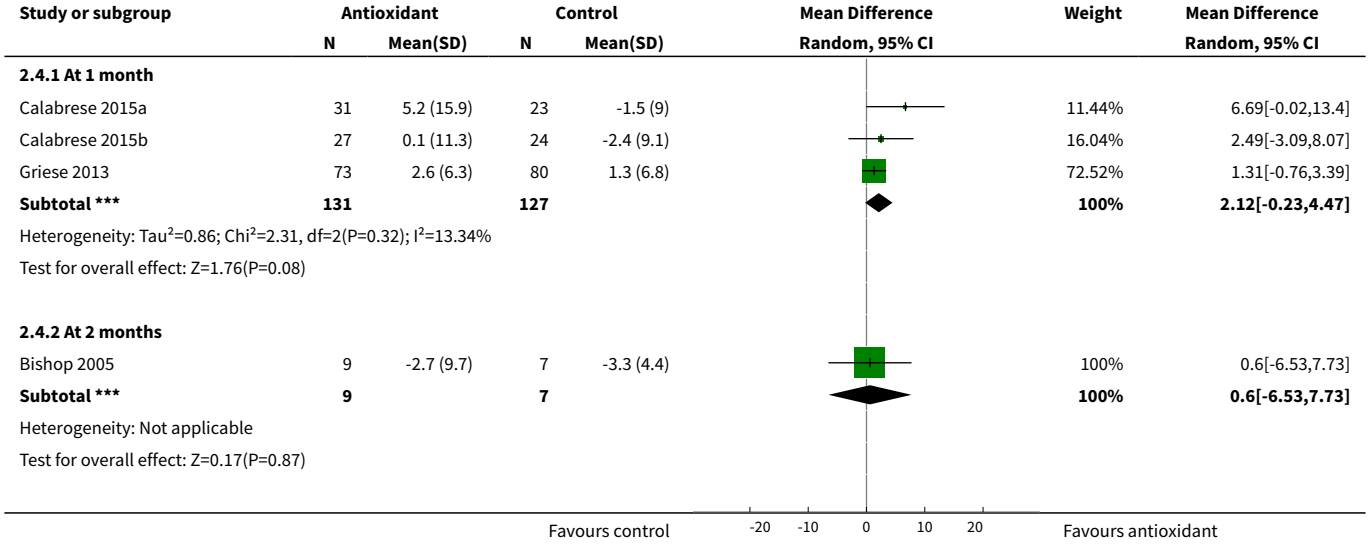


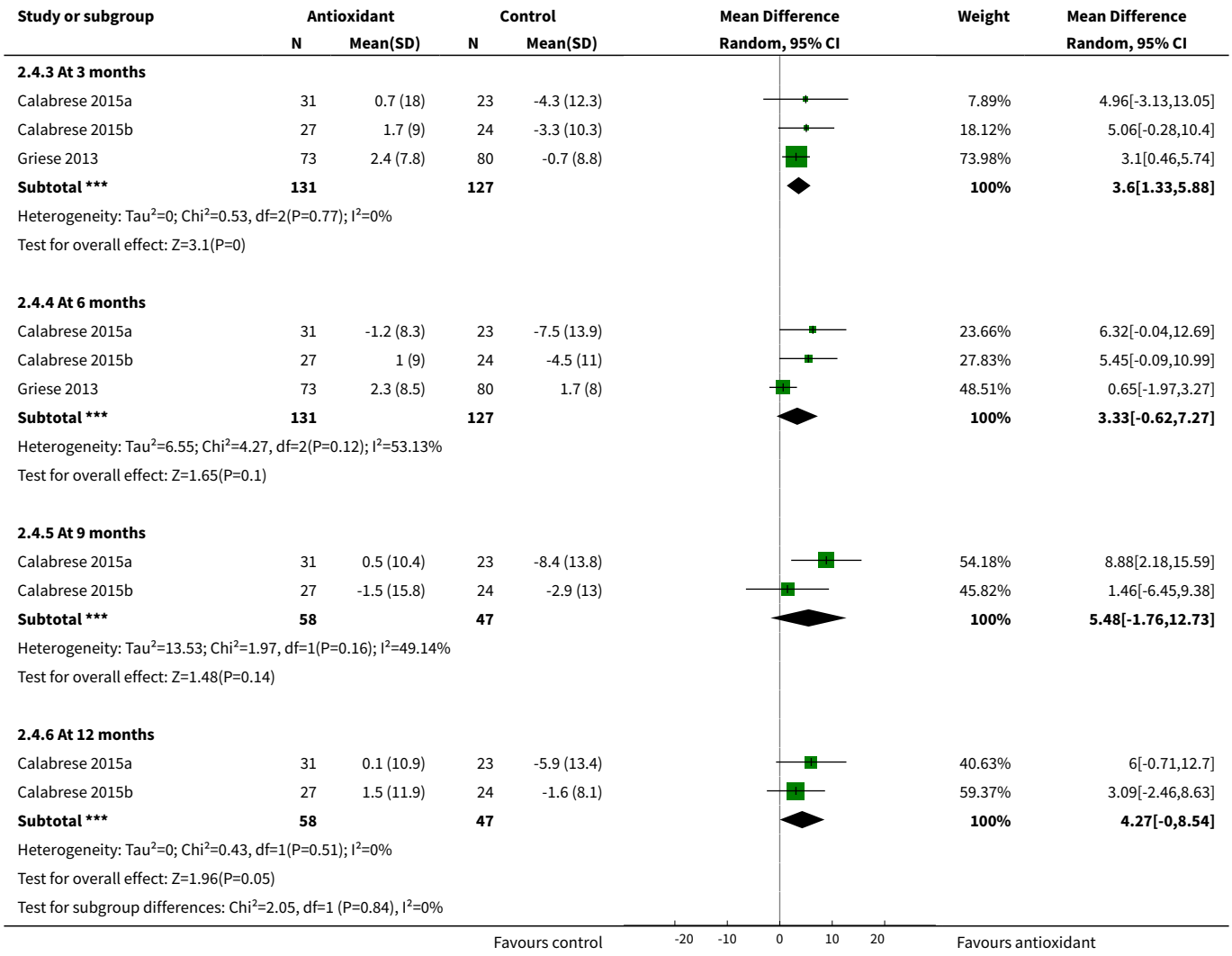
Analysis 2.3. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 3 Lung function FVC (L) (change from baseline).



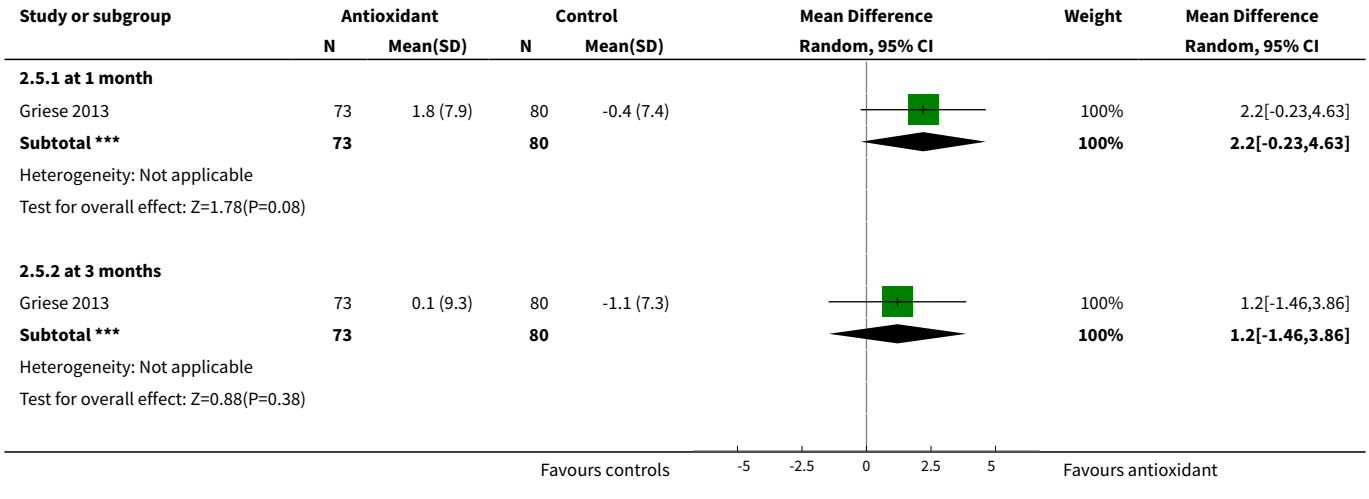


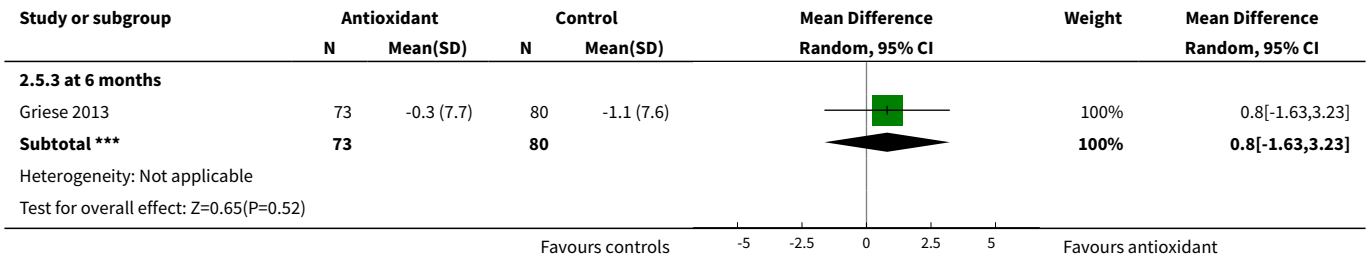
Analysis 2.4. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 4 Lung function FVC (% predicted) (change from baseline).



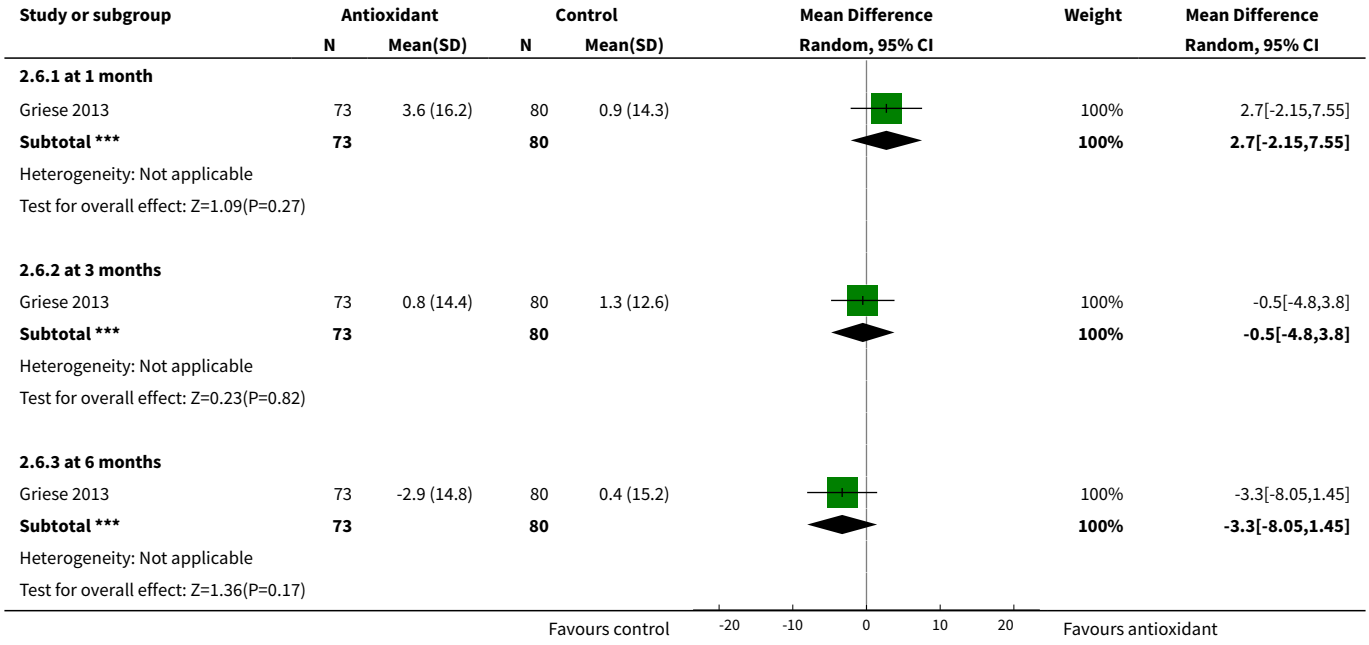


Analysis 2.5. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 5 QoL total score (change from baseline).

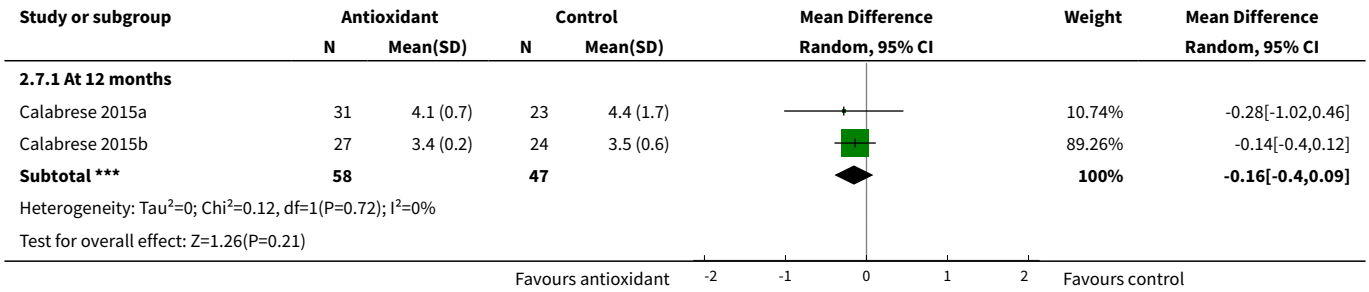




Analysis 2.6. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 6 QoL respiratory score (change from baseline).

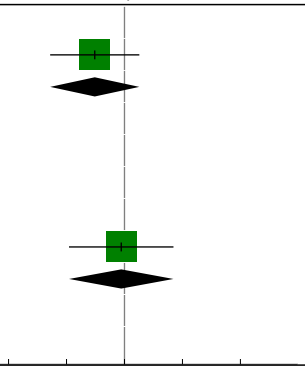


Analysis 2.7. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 7 Oxidative stress markers in exhaled breath condensate: H_2O_2 (µM).



Analysis 2.8. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 8 Sputum oxidative stress: lipid peroxidation (8-isoprostan).

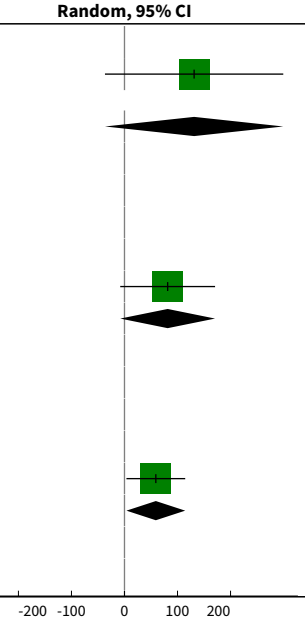
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
2.8.1 At 3 months							
Griese 2013	18	-8.5 (108.2)	24	42.8 (146.2)		100%	-51.3[-128.22,25.62]
Subtotal ***	**18**		**24**			**100%**	**-51.3[-128.22,25.62]**
Heterogeneity: Not applicable							
Test for overall effect: Z=1.31(P=0.19)							
2.8.2 At 6 months							
Griese 2013	19	-2.9 (167.2)	23	2.7 (121.5)		100%	-5.6[-95.7,84.5]
Subtotal ***	**19**		**23**			**100%**	**-5.6[-95.7,84.5]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.12(P=0.9)							



Favours antioxidant — Favours control

Analysis 2.9. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 9 Sputum antioxidant status: free glutathione in sputum (pM).

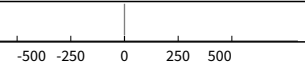
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
2.9.1 At 1 month							
Griese 2013	25	129.5 (428.4)	30	-1.8 (22.7)		100%	131.3[-36.81,299.41]
Subtotal ***	**25**		**30**			**100%**	**131.3[-36.81,299.41]**
Heterogeneity: Not applicable							
Test for overall effect: Z=1.53(P=0.13)							
2.9.2 At 3 months							
Griese 2013	23	80.5 (218.2)	24	-0.9 (16)		100%	81.4[-8.01,170.81]
Subtotal ***	**23**		**24**			**100%**	**81.4[-8.01,170.81]**
Heterogeneity: Not applicable							
Test for overall effect: Z=1.78(P=0.07)							
2.9.3 At 6 months							
Griese 2013	23	56.6 (134.2)	27	-2.5 (21.4)		100%	59.1[3.68,114.52]
Subtotal ***	**23**		**27**			**100%**	**59.1[3.68,114.52]**
Heterogeneity: Not applicable							
Test for overall effect: Z=2.09(P=0.04)							



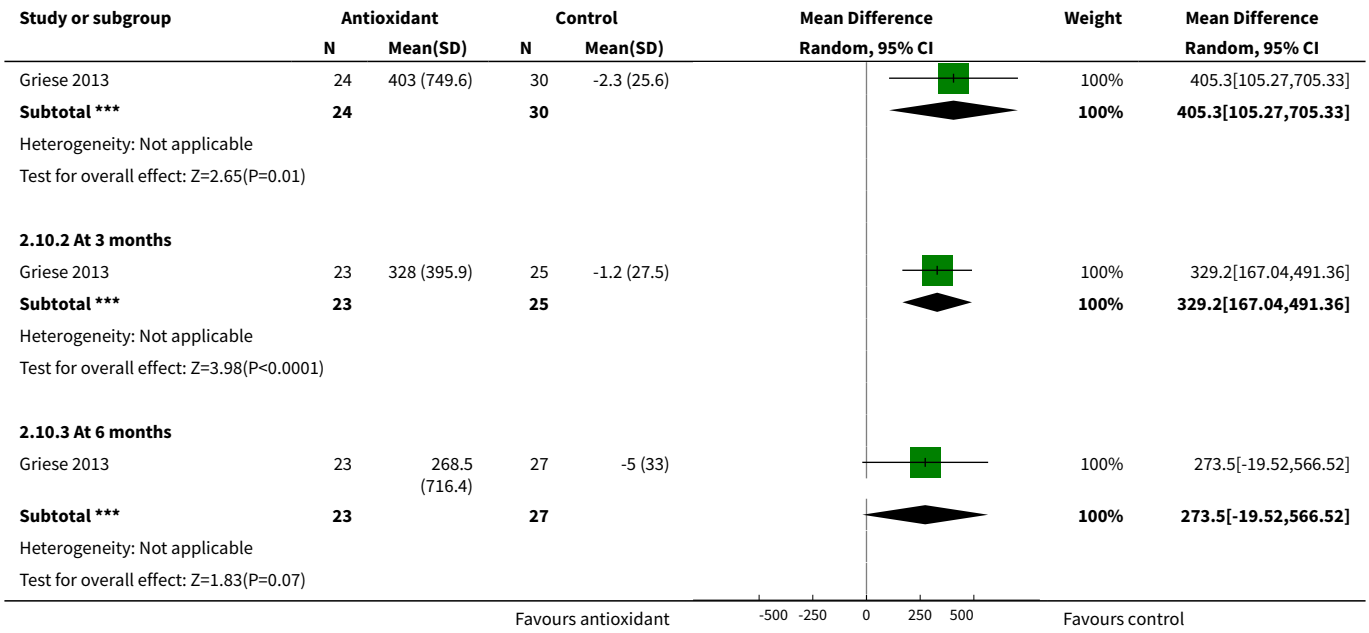
Favours antioxidant — Favours control

Analysis 2.10. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 10 Sputum antioxidant status: total glutathione in sputum (pM).

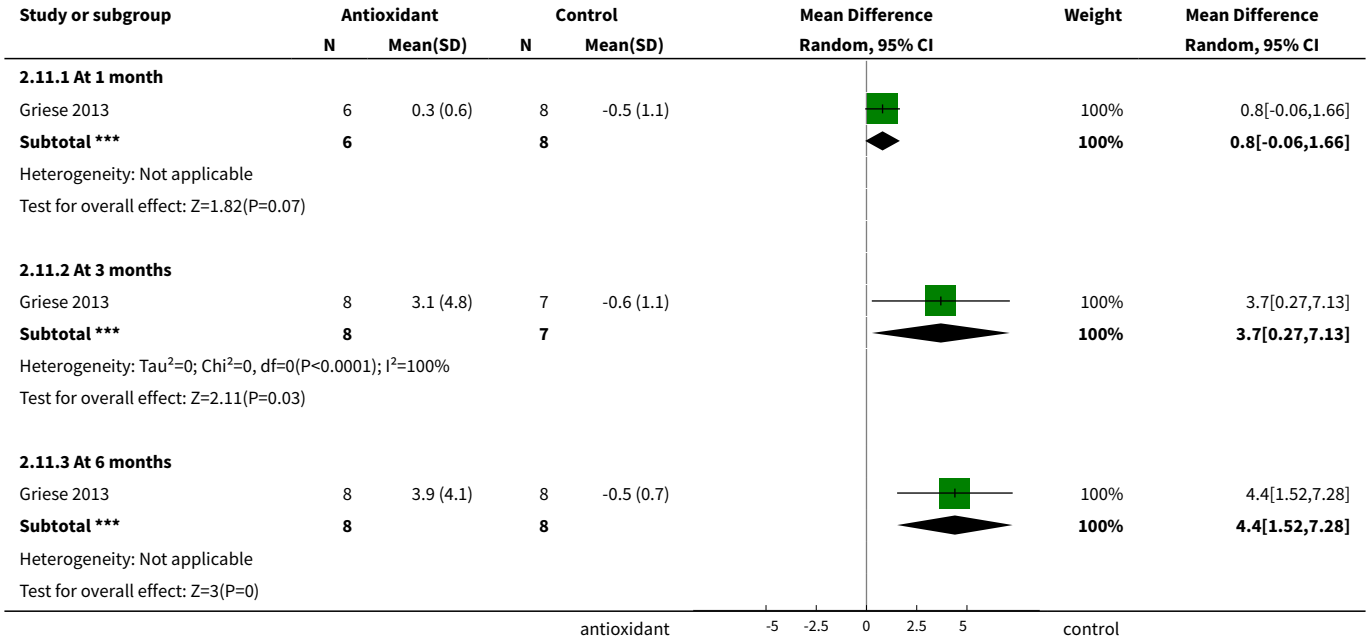
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
2.10.1 At 1 month							



Favours antioxidant — Favours control

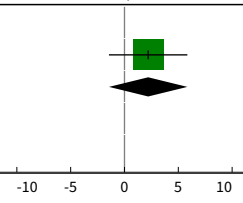


Analysis 2.11. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 11 Sputum antioxidant status: glutathione in sputum neutrophils (MFI).



Analysis 2.12. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 12 Plasma antioxidant status: free glutathione (pM).

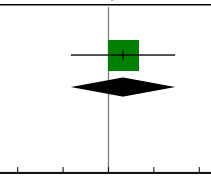
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
2.12.1 At 6 months							
Griese 2013	25	2.4 (9.2)	32	0.2 (1.1)		100%	2.2[-1.44,5.84]
Subtotal ***	**25**		**32**			**100%**	**2.2[-1.44,5.84]**
Heterogeneity: Not applicable							
Test for overall effect: Z=1.18(P=0.24)							



Favours antioxidant — Favours control

Analysis 2.13. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 13 Plasma antioxidant status: total glutathione (pM).

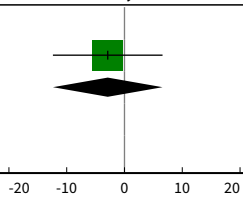
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
2.13.1 At 6 months							
Griese 2013	25	0.2 (4)	32	-0.6 (6.9)		100%	0.8[-2.07,3.67]
Subtotal ***	**25**		**32**			**100%**	**0.8[-2.07,3.67]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.55(P=0.59)							



Favours antioxidant — Favours control

Analysis 2.14. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 14 Plasma antioxidant status: glutathione in blood neutrophils (MFI).

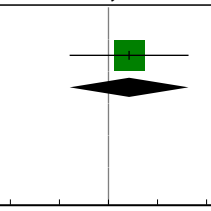
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
2.14.1 At 6 months							
Griese 2013	4	2.6 (5.1)	9	5.5 (12.3)		100%	-2.9[-12.39,6.59]
Subtotal ***	**4**		**9**			**100%**	**-2.9[-12.39,6.59]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.6(P=0.55)							



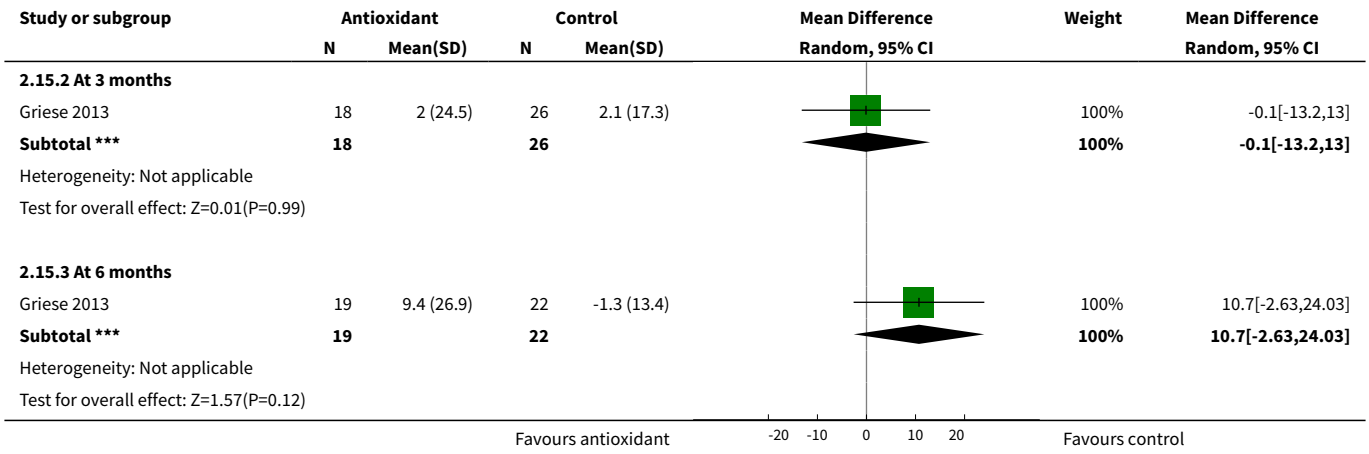
Favours antioxidant — Favours control

Analysis 2.15. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 15 Sputum oxidative stress: protein carbonyls (U).

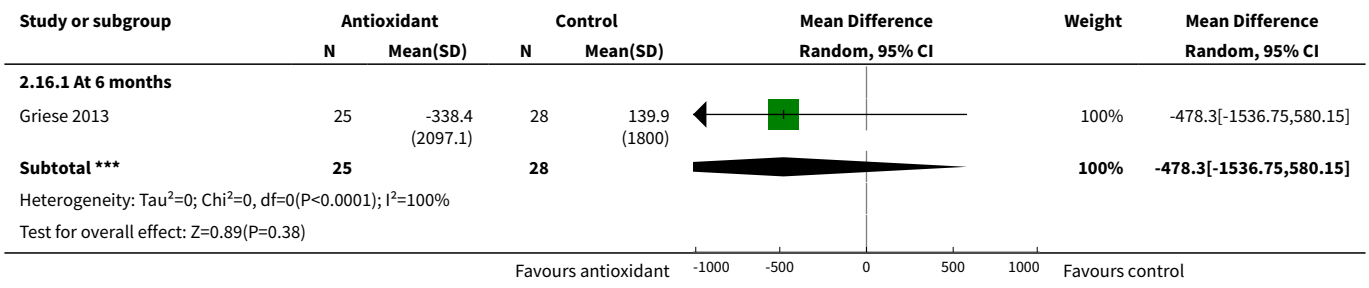
Study or subgroup	Antioxidant N	Antioxidant Mean(SD)	Control N	Control Mean(SD)	Mean Difference Random, 95% CI	Weight	Mean Difference Random, 95% CI
2.15.1 At 1 month							
Griese 2013	21	6.1 (20.3)	28	1.9 (22.9)		100%	4.2[-7.92,16.32]
Subtotal ***	**21**		**28**			**100%**	**4.2[-7.92,16.32]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.68(P=0.5)							



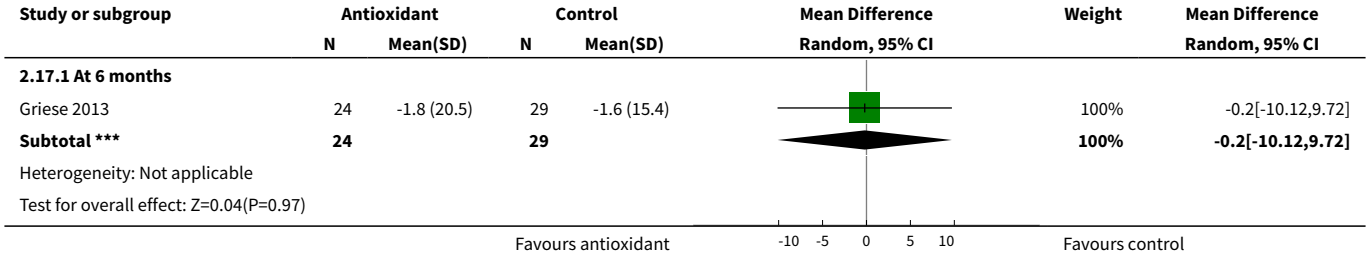
Favours antioxidant — Favours control



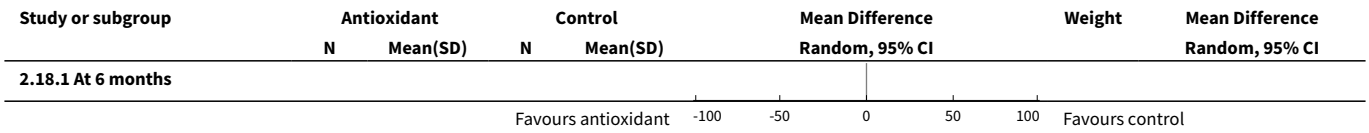
Analysis 2.16. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 16 Local inflammation: cytokines in sputum IL-8 (pg/mL).

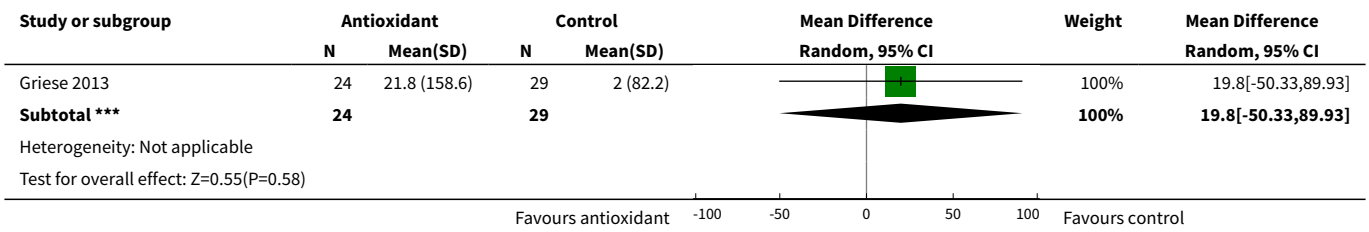


Analysis 2.17. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 17 Local inflammation: cytokines in sputum IL-10 (pg/mL).

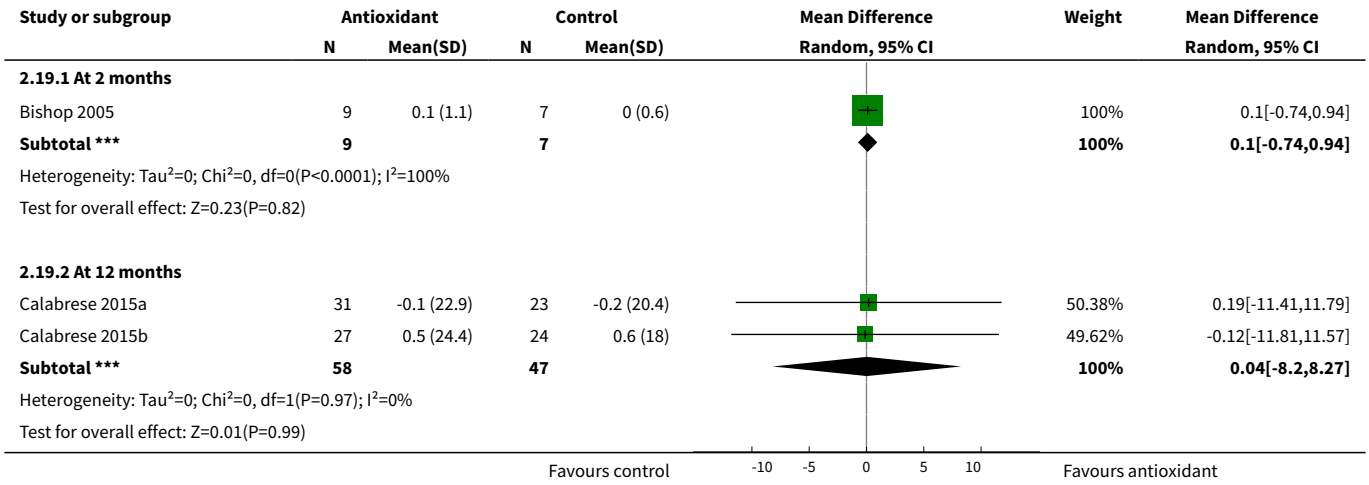


Analysis 2.18. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 18 Local inflammation: cytokines in sputum TNF-α (pg/mL).

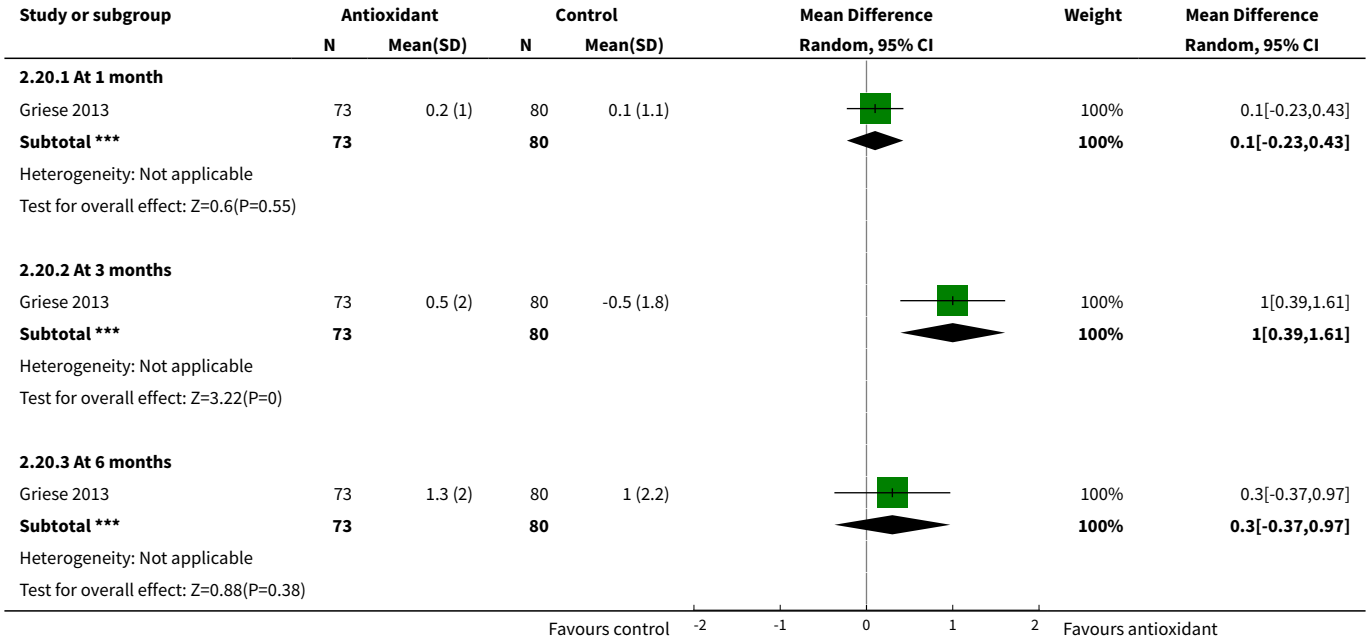




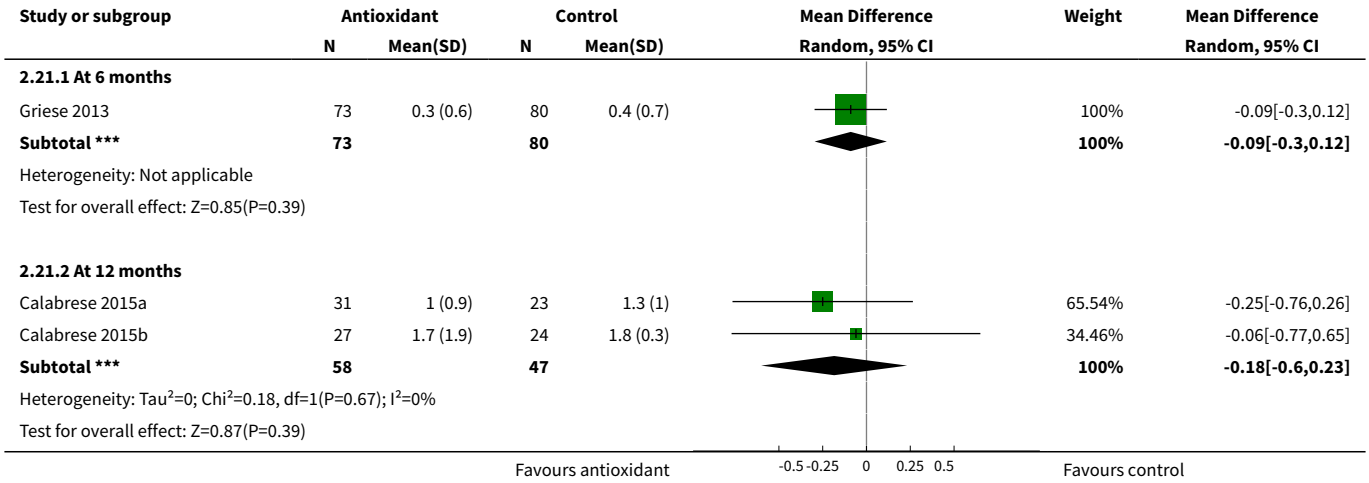
Analysis 2.19. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 19 Nutritional status: BMI (change from baseline).



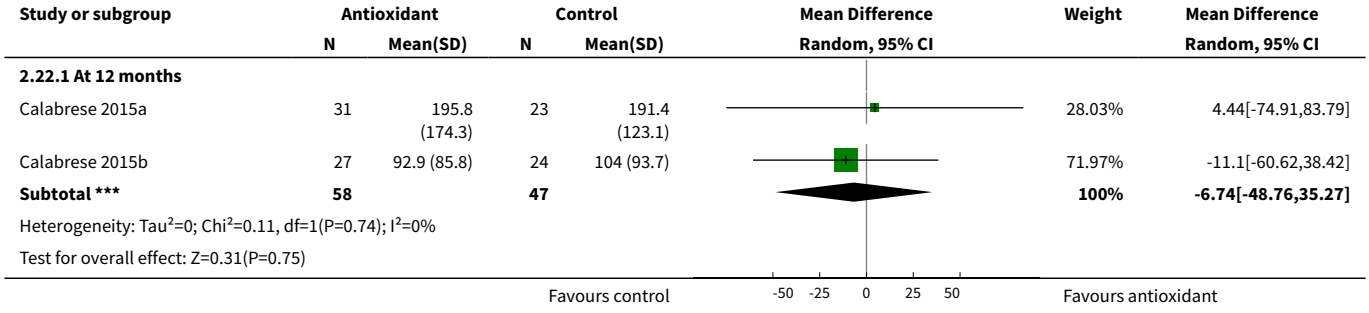
Analysis 2.20. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 20 Nutritional status: weight (kg).



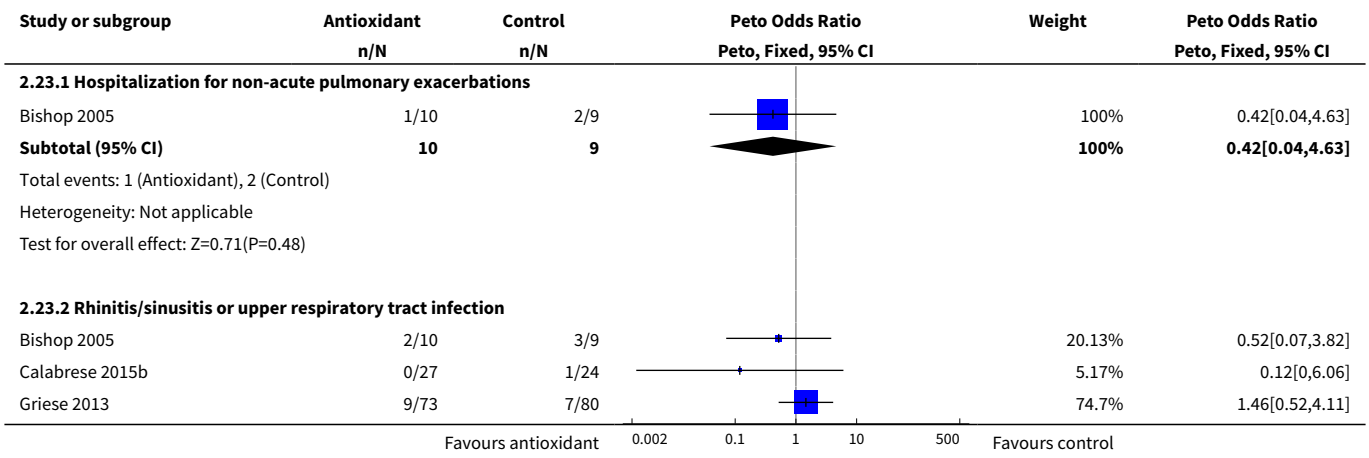
Analysis 2.21. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 21 Number of pulmonary exacerbations during the study.

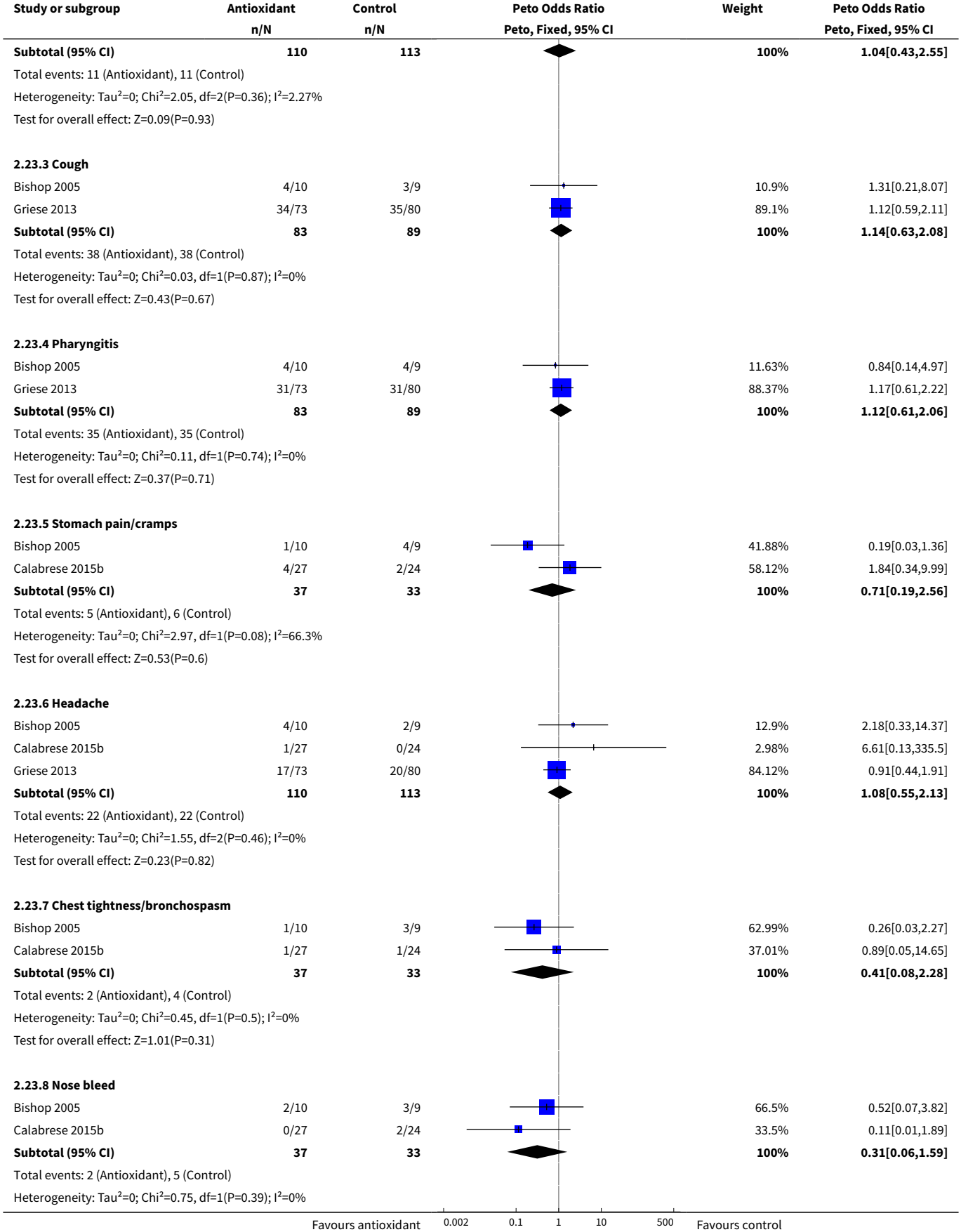


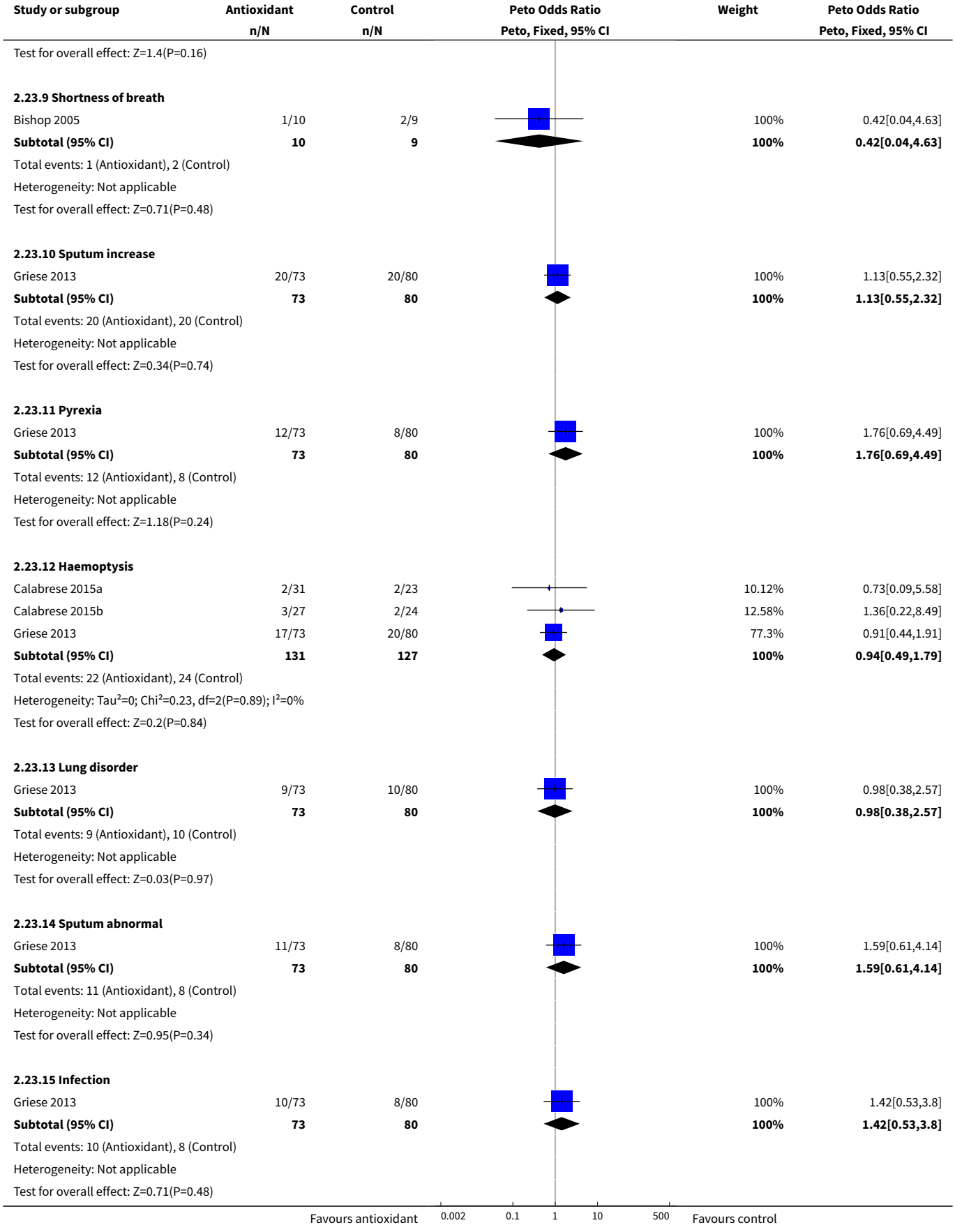
Analysis 2.22. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 22 Time to first pulmonary exacerbation (days).

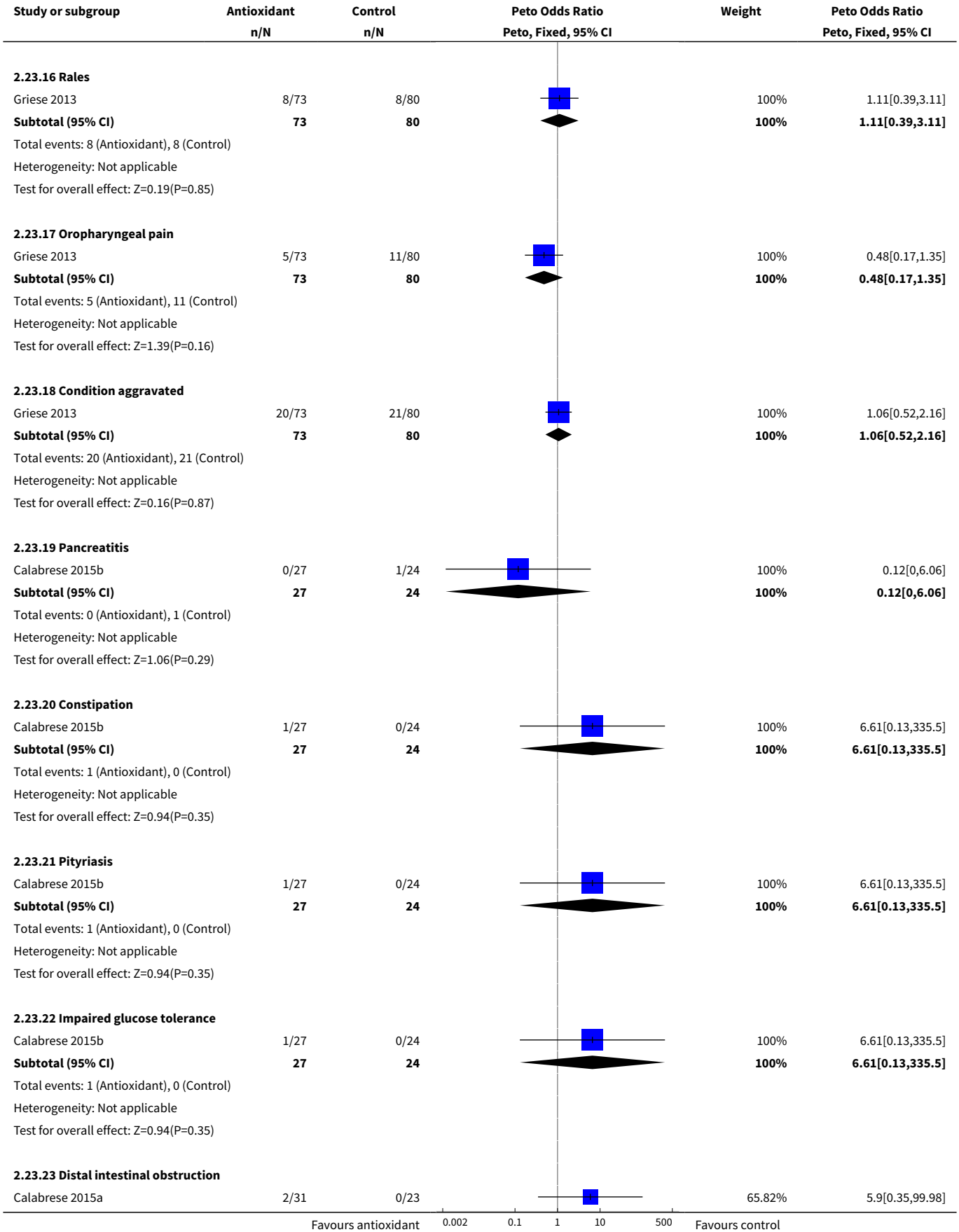


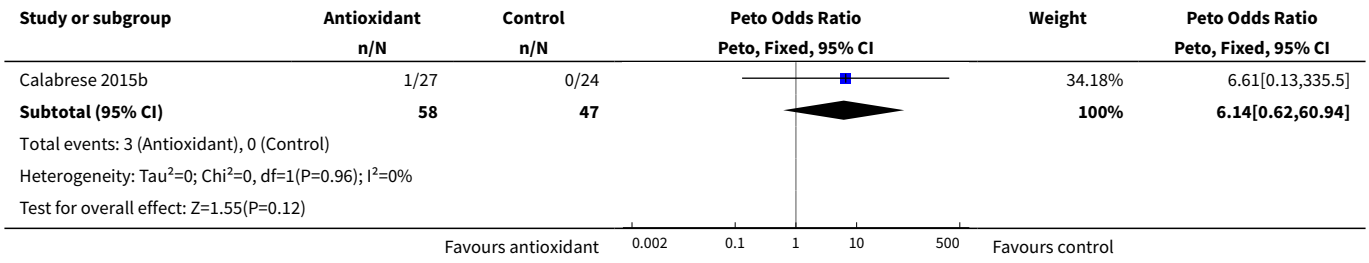
Analysis 2.23. Comparison 2 Inhaled antioxidant (glutathione) versus control, Outcome 23 Adverse events.











ADDITIONAL TABLES

Table 1. Time points for reporting data in studies of oral supplementation

Study	Time point reported							
	1 month	2 months	3 months	4 months	5 months	6 months	9 months	12 months
Conrad 2015			✓			✓		
Dauletbaev 2009			✓					
Götz 1980	✓							
Harries 1971	✓							
Homnick 1995b								✓
Keljo 2000			✓					
Levin 1961		✓				✓		
Mitchell 1982			✓					
Portal 1995a					✓			
Ratjen 1985			✓					
Renner 2001			✓			✓		
Sagel 2018	✓			✓				
Stafanger 1988			✓					
Stafanger 1989			✓					
Visca 2015			✓			✓		
Wood 2003		✓						

Table 2. Time points for reporting data in studies of inhaled supplementation

Study	Time point reported

Table 2. Time points for reporting data in studies of inhaled supplementation *(Continued)*

	1 month	2 months	3 months	4 months	5 months	6 months	9 months	12 months
Bishop 2005		✓						
Calabrese 2015a; Calabrese 2015b	✓		✓			✓	✓	✓
Griese 2013	✓		✓			✓		
Howatt 1966				✓				

Table 3. Improvement in plasma vitamin E levels (µmol/L) after supplementation with antioxidants in people with CF

Supplement	Time point	Result
Fat-soluble vitamin E	1 month (Harries 1971)	MD 13.47 µmol/L (95% CI 9.05 to 17.89)
Water-miscible vitamin E	1 month (Harries 1971)	MD 26.7µmol/L (95% CI 15.90 to 37.50)
	2 months (Levin 1961)	MD 11.61 µmol/L (95% CI 4.31 to 18.91)
	6 months (Levin 1961)	MD 19.73 µmol/L (95% CI 12.48 to 26.98)
Combined supplement	2 months (Wood 2003)	MD 10.20 µmol/L (95% CI 5.21 to 15.19)
Oral GSH	6 months (Visca 2015)	MD 4.26 µmol/L (95% CI 2.03 to 6.49)
Antioxidant-enriched multivitamin	1 month (Sagel 2018)	MD -3.48 µmol/L (95% CI -8.01 to 1.05)
	4 months (Sagel 2018)	MD -1.86 µmol/L (95% CI -6.36 to 2.64)

CF: cystic fibrosis
CI: confidence interval
GSH: glutathione
MD: mean difference

Table 4. Changes in plasma levels of cytokines IL-6 (pg/mL) and TNF-α (pg/mL) after 3 months of supplementation with vitamin E (Keljo 2000)

Subgroup	IL-6 (pg/mL)	TNF-α (pg/mL)
FEV_1 > 85% and no DNase	MD -2.02 (95% CI -4.63 to 0.59)	MD -1.37 (95% CI -3.61 to 0.87)
FEV_1 > 85% and no DNase	MD -0.31 (95% CI -4.03 to 3.41)	MD 0.33 (95% CI -0.49 to 1.15)
FEV_1 range 70% - 85% and DNase	MD -0.24 (95% CI -3.80to 3.32)	MD -0.94 (95% CI -1.61 to -0.26)

CI: confidence interval
FEV_1: forced expiratory volume in 1 second
MD: mean difference

Table 5. CF Quality of life scores stratified according to age

Study ID	Age	GSH		Placebo	
		Baseline mean (SD)	At 12 months mean (SD)	Baseline mean (SD)	At 12 months mean (SD)
Calabrese 2015a	Over 18 years	882.75 (147.63)	891.69 (165.09)	871.18 (124.56)	879.47 (139.9)
Calabrese 2015b	14 to 18 years	949.23 (82.94)	955.34 (94.95)	1044.5 (65.53)	1049.12 (76.87)
	12 - 13 years	506.12 (34.15)	527.39 (132.25)	679.24 (55.79)	705.55 (40.93)
	12 - 13 years (parents)	634.93 (122.59)	643.06 (75.86)	975.34 (57.05)	959.79 (130.95)
	6 to 11 years	544.21 (127.59)	925.16 (104.95)	573.64 (103.19)	574.95 (107.73)
	6 to 11 years (parents)	771.79 (231.05)	779.59 (222.27)	745.23 (164.84)	767.59 (154.02)

GSH: glutathione
SD: standard deviation

APPENDICES

Appendix 1. Additional search strategy: PubMed (NLM) (1950 to August 2013)

Database or Resource	Strategy
PubMed (1946 - 31 May 2016)	#1 randomized controlled trial [pt]
	#2 controlled clinical trial [pt]
	#3 randomized [tiab]
	#4 placebo [tiab]
	#5 drug therapy [sh]
	#6 randomly [tiab]
	#7 trial [tiab]
	#8 groups [tiab]
	#9 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8
	#10 animals [mh] NOT humans [mh]
	#11 #9 NOT #10
	#12 cystic fibrosis OR mucoviscidosis OR “fibrocystic disease of the pancreas”

(Continued)

	#13 antioxidant* OR vitamin e OR carotene OR betacarotene OR tocopherol OR tocotrienol OR vitamin C OR "ascorbic acid" OR ascorbate OR ascorbicum OR selenium OR magnorbin OR GSH OR Glutathione OR NAC OR Acetylcysteine OR Cysteine OR oxidative stress #14 #11 AND #12 AND #13 NOTE: Lines #1 to #11 are the Cochrane Highly Sensitive Search Strategy for identifying randomized trials in MEDLINE: sensitivity-maximizing version (2008 revision); PubMed format
ISRCTN registry	BASIC SEARCH (cystic fibrosis OR mucoviscidosis) AND (antioxidant OR "vitamin e" OR carotene OR betacarotene OR tocopherol OR tocotrienol OR "vitamin c" OR "ascorbic acid" OR ascorbate OR ascorbicum OR selenium OR magnorbin OR GSH OR Glutathione OR NAC OR Acetylcysteine OR Cysteine OR oxidative)
ClinicalTrials.gov	ADVANCED SEARCH FORM CONDITION OR DISEASE: "cystic fibrosis" OR mucoviscidosis OTHER TERMS: antioxidant OR "vitamin E" OR "vitamin C" OR "ascorbic acid" OR ascorbate OR ascorbicum OR carotene OR betacarotene OR selenium OR GSH OR Glutathione OR NAC OR Acetylcysteine OR Cysteine OR tocopherol OR tocotrienol OR magnorbin OR oxidative STUDY TYPE: Interventional Studies (Clinical Trials) STUDY RESULTS: All studies
WHO International Clinical Trials Registry Platform (ICTRP)	ADVANCED SEARCH FORM TITLE: cystic fibrosis OR mucoviscidosis INTERVENTION ["Without Synonyms" box ticked]: antioxidant OR "vitamin E" OR "vitamin C" OR "ascorbic acid" OR ascorbate OR ascorbicum OR carotene OR betacarotene OR selenium OR GSH OR Glutathione OR NAC OR Acetylcysteine OR Cysteine OR tocopherol OR tocotrienol OR magnorbin OR oxidative RECRUITMENT STATUS: All

Appendix 2. Search strategies for online trials registries

Trials registry	Search terms	Search date
Register of international standard randomised controlled trial numbers (ISRCTN) (www.isrctn.com/)	'cystic fibrosis and antioxidants' OR 'cystic fibrosis and oxidative stress'	16 July 2018
WHO International Clinical Trials Registry Platform (WHO ICTRP)	'cystic fibrosis and antioxidants' OR 'cystic fibrosis and oxidative stress'	16 July 2018
ClinicalTrials.gov (clinicaltrials.gov)	'cystic fibrosis and antioxidants' OR 'cystic fibrosis and oxidative stress'	16 July 2018

Appendix 3. Additional search strategy CINAHL Plus with full text (EBSCO) (1937 to December 2007)

Database or Resource	Strategy
CINAHL Plus with full text EBSCO (1937 to December 2007)	MJ cystic fibrosis OR MJ mucoviscidosis OR MJ fibrocystic disease of pancreas AND "vitamin E" OR tocopherol OR tocotrienol OR alpha-tocopherol OR beta-carotene OR betacarotene OR "vitamin C" OR "ascorbic acid" OR "l-ascorbic acid" OR "ferrous ascorbate" OR "hybrin magnesium ascorbicum" OR magnorbin OR "sodium ascorbate" OR selenium OR antioxidant AND TX control* trial* or TX intention to treat or TX sham Or TX mask* or TX placebo* or TX double blind Or TX single blind Or TX triple blind or TX efficacy Or TX effectiveness or TX random* or PT critical path Or PT care plan Or PT protocol or PT nursing interventions or PT practice guidelines Or PT systematic review or PT research Or PT clinical trial or (MH "Outcomes (Health Care)+") or (MH "Professional Practice, Research-Based+") or (MH "Research") or (MH "random sample+") or (MH "community trials") or (MH "experimental studies") or (MH "study design") or (MH "comparative studies") or (MH "placebos") or (MH "sample size") or (MH "random assignment") or (MH "clinical trials+") or (MH "patient selection") or (MH "Crossover Design") or (MH "Meta Analysis") or (MH "Research Methodology") or (MH "Clinical Research+") or (MH "Reproducibility of Results") or (MH "Pilot Studies")
AMED Ovid (1985 to December 2007)	1.exp Cystic Fibrosis/ 2.exp Antioxidant/ or alpha tocopherol.mp. or vitamin E.mp. or exp Ascorbic Acid/ or vitamin C.mp. or Beta Carotene.mp. or exp Selenium/ 3.1 AND 2

Appendix 4. Additional search strategy: AMED (Ovid) (1985 to December 2007)

Search strategy
1.exp Cystic Fibrosis/ 2.exp Antioxidant/ or alpha tocopherol.mp. or vitamin E.mp. or exp Ascorbic Acid/ or vitamin C.mp. or Beta Carotene.mp. or exp Selenium/ 3.1 AND 2

WHAT'S NEW

Date	Event	Description
3 October 2019	New citation required and conclusions have changed	A new author, Sherie Smith, has joined the author team. The inclusion of new studies has provided additional evidence on the effects of glutathione.
3 October 2019	New search has been performed	A search of the Cochrane Cystic Fibrosis and Genetic Disorders Review Group's Cystic Fibrosis Register (using the term 'antioxidant' and ensuring that studies of glutathione or N-acetylcys-

Date	Event	Description
		teine (NAC) were identified) found 51 new references which were possibly eligible for inclusion in the review. Three references (one abstract and the full paper with supplementary materials) referred to an already included study, which was previously only published as an abstract (Visca 2015). One reference was to a second already included study (Griese 2013). We included a total of 10 new studies (14 references) with 11 data sets in the present update (Calabrese 2015a; Calabrese 2015b; Conrad 2015; Dauletbaev 2009; Götz 1980; Howatt 1966; Mitchell 1982; Ratjen 1985; Sagel 2018; Stafanger 1988; Stafanger 1989). Two of these studies have now been published, having previously been listed as ongoing studies (NCT00809094; NCT01859390); there are three new references related to the Conrad study and three new references related to the Sagel study (Conrad 2015; Sagel 2018). Three references were additional references to a study which was previously excluded (originally only available as an abstract with the ID Casale 2012, but which has now been included (with two data sets) (Calabrese 2015a; Calabrese 2015b). The original abstract implied that the study was of only a single inhalation of glutathione, but the published full paper clarifies that this is not the case and that the study is eligible for inclusion. Four references were to two newly included studies by Stafanger (two references each) (Stafanger 1988; Stafanger 1989) and two references were to the Ratjen study (Ratjen 1985). Four of the newly included studies each had a single reference (Dauletbaev 2009; Götz 1980; Howatt 1966; Mitchell 1982). Two studies with one reference each are listed as excluded (Khorasani 2009; Sharma 2016). Two studies with one reference each have been listed as 'Awaiting assessment' pending further information (Tirouvanziam 2005; Tirouvanziam 2006). The remaining 29 references did not fulfil the inclusion criteria and were excluded on title alone and not listed in this updated review. A summary of findings table for each comparison presented in the review has been added.

HISTORY

Protocol first published: Issue 2, 2008
Review first published: Issue 12, 2010

Date	Event	Description
7 August 2014	New citation required and conclusions have changed	A new review team has taken on this review. The inclusion of the new studies means that information on clinically relevant outcome measures, such as lung function (forced expiratory volume at one second) is now included in the review.
7 August 2014	New search has been performed	A search of the Group's Cystic Fibrosis Trials Register identified 12 references to eight studies.

Date	Event	Description
		Three of the eight studies have been included in the updated review (Bishop 2005; Griese 2013; Visca 2015). Three additional studies previously listed as 'Awaiting classification' have now also been included (Harries 1971; Levin 1961; Keljo 2000). The fourth study previously listed as 'Awaiting classification' has now been excluded (Jacquemin 2009). According to the modified inclusion criteria (more than a single administration of antioxidants) one of the studies which was included in the original review, has now been excluded (Homnick 1995a).
22 May 2012	Amended	Contact details updated.
12 May 2008	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

Original review

Task	Details
Guarantee review	SV is the guarantor of this review.
Background to review	LS and NB performed previous work that was the foundation of the current review.
Design of review	LS and SV conceived this review and secured funding for it. LS led the design and ongoing coordination of this review with oversight from SV. SV, DA, JJ and NB provided general guidance and a methodological perspective on this review on an ongoing basis. LS developed the additional search strategies for this review including 'grey literature' (i.e. literature which is not easily accessible through electronic databases).
Review process	LS carried out the searches and organized the retrieval of papers for this review. LS and DA screened retrieved papers against inclusion criteria for this review. SV settled disagreements between LS and DA regarding included studies for this review. LS and DA independently appraised the quality of papers and extracted data from these papers for this review. LS wrote to authors of included studies for additional information for this review. LS managed data for the review including entering data into RevMan and analysing the data with the assistance of a statistician if needed. LS and SV interpreted data for this review. LS wrote the review with revisions suggested by NB, JJ, SV.

Updates from 2014 onwards

Task	Details
Guarantee review	JL is the guarantor of this review.
Background to review	OC and JL performed previous work on antioxidants and cystic fibrosis.
Design of review	OC and JL conceived this review and secured funding for it. OC led the design and ongoing coordination of this review with oversight from JL.
Review process	OC and JL organized the retrieval of papers for this review. OC and JL independently screened the retrieved papers against inclusion criteria for this review. OC, SS and JL independently appraised

the quality of papers and extracted data from these papers for this review. OC wrote to authors of included studies for additional information for this review. OC, SS and JL managed data for the review including entering data into RevMan and analysing the data. OC, SS and JL interpreted data for this review. OC wrote the review with revisions suggested by SS and JL.

DECLARATIONS OF INTEREST

Original review

All authors: none known.

Updates from 2014 onwards

All authors: none known.

SOURCES OF SUPPORT

Internal sources

- Department of International Health, Immunology and Microbiology, Costerton Biofilm Center, University of Copenhagen, Denmark.

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Original review

Each antioxidant micronutrient or unique combination of micronutrients were analysed as separate subgroups within meta-analyses since their mechanisms of action are different.

Quality assessment was conducted using Cochrane's newly adopted risk of bias (RoB) tool rather than the Jadad scale and as such proposed sensitivity analyses were to be based on RoB assessments.

Sensitivity analysis was intended for all outcomes, rather than just lung function, oxidative stress and inflammatory stress outcomes as stated in the protocol.

Three secondary outcomes were revised after the review process began. Categories of oxidative stress outcomes were revised and pulmonary exacerbations were not specifically collected since this data appeared in the literature as "days of antibiotic therapy".

After statistical advice from the statistical peer reviewer and the CFGD Group's statistical editor, we now plan to present results for adverse events using Peto OR rather than the risk difference.

Update 2014

A new author team has taken on this review and expanded the scope of the review to include glutathione.

The different routes of administration are presented separately.

The inclusion criteria have been modified so that doses need to be more than a single administration of any antioxidant.

Definition of CF diagnosis removed.

Update 2019

The current author team has updated the review and added N-acetylcysteine as a precursor of glutathione as an antioxidant supplement. Summary of findings tables (one for each comparison presented in the review) have been added.

NOTES

At 2014 update

Three of the five studies that were listed as 'Awaiting classification' in the initial version of the review were included in the analysis at the 2014 update (Harries 1971; Keljo 2000; Levin 1961). A further study which was also listed as 'Awaiting classification' in the initial version of the review was a single dose bioavailability study and did not meet the inclusion criteria and so was excluded (Jacquemin 2009). One study

which was included in the first version of the review, was excluded as it was presenting bioavailability data after a single administration (Homnick 1995a).

INDEX TERMS

Medical Subject Headings (MeSH)

Administration, Inhalation; Administration, Oral; Antioxidants [*therapeutic use]; Ascorbic Acid [therapeutic use]; Cystic Fibrosis [*drug therapy]; Micronutrients [*therapeutic use]; Oxidative Stress; Quality of Life; Randomized Controlled Trials as Topic; Selenium [therapeutic use]; Vitamin E [therapeutic use]; Vitamins [therapeutic use]

MeSH check words

Adult; Child; Humans